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Subject: Clinical Review of BLA 99-0903
Genentech, Inc.; Tenecteplase

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To: BLA 99-0903 File

This document is the Medical Officer Clinical Review for BLA-99-0903

Sponsor: Genentech, Inc.
Product: Tenecteplase (TNK)
Proposed Indication: treatment of acute myocardial infarction
Proposed Regimen: weight-adjusted, 30-50 mg IV bolus
Dates of Submissions:
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Supplemental Information:
September 10, 1999 – 365h Item 19 (Other) – Financial Disclosure
September 17, 1999 – ASSENTII Study Conduct Supplement
September 20, 1999 – Financial Disclosure – Due diligence
September 24, 1999 – Validation of non-parametric analyses macro
October 8, 1999 – Request for Pediatric Waiver 21 CFR 601.27(c)(2)(ii)
October 26, 1999 – Amended Labeling (name change to “TNKase”)
November 15, 1999 – 365h Item 19 (Other) Financial Disclosure
March 3, 2000 – Inclusion/Exclusion Data for study N0747g
March 14, 2000 – 365h Item 19 (Other) Financial Disclosure
March 15, 2000 – Corrected Tables for study N0747g
March 27, 2000 – Responses to CBER questions
March 31, 2000 – Revised Package Insert
April 26, 2000 – Antibody incidence in TIMI 10B; other information

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Overview

Proposed TNK Indication

The proposed TNK indication is: "TNKase is indicated for use in the reduction of mortality associated with acute myocardial infarction."

This indication statement is a departure from those of other licensed thrombolytic agents for AMI, because it lacks a statement regarding left ventricular function.
Reviewer's Comment(s): Left ventricular function was not systematically assessed in the phase 2 and 3 clinical development of the product.

Scope of This Review

The initial phase 1 clinical trial, N0647g (TIMI 10A), was an open-label, uncontrolled dose-finding study of TNK in AMI, and is not reviewed extensively. The main focus of this review is the analysis of subsequent phase 2 and 3 studies.

Two phase 2 studies were conducted concurrently: N0660g (TIMI 10B) and N0683g (ASSENT I):

- Study N0660g was an 880-subject, randomized, controlled, open-label, angiographic trial comparing single IV bolus administration of 3 different doses of TNK with accelerated t-PA. Endpoints were primarily angiographic patency of the infarct-related artery (IRA).
- Study N0683g was a 3301-subject, randomized open-label, dose-ranging, safety trial to evaluate the risk of ICH associated with TNK at doses of 30, 40 and 50 mg. There was no non-TNK-treated control group.

The pivotal phase 3 trial, N0747g (ASSENT II), was a 17,005-subject, randomized, double-blind, double-dummy, parallel-group, international trial of a single IV bolus of TNK versus accelerated t-PA in AMI. The primary efficacy objective was to demonstrate no unacceptable inferiority in 30-day mortality on the primary endpoint of 30-day mortality.

Abbreviations Used in This Review

ACT	activated clotting time
[redacted]	[redacted]
Activase ®	Genentech brand name for Alteplase
Alteplase	specific generic name of rt-PA manufactured by Genentech or Boehringer Ingelheim
AMI	acute myocardial infarction
AUC	area under the time-concentration curve, determined over one of two time intervals:
	1) from time zero to infinity (total AUC)
	2) from time zero or a specific early timepoint to a late timepoint (truncated AUC)
ASSENT I trial	assessment of the safety of a new thrombolytic: Tenecteplase
ASSENT II trial	assessment of the safety and efficacy of a new thrombolytic agent: Tenecteplase
AUC/D	dose-adjusted area under the time-concentration curve
$AUC_{2\text{-}90}$	truncated AUC from 2-90 minutes following IV bolus Tenecteplase administration
BMI	body mass index
BP	blood pressure
BPd	diastolic blood pressure
BPs	systolic blood pressure
C(0)	predicted plasma concentration at time zero

CABG	coronary artery bypass graft (surgery)
CI	confidence interval
CL	plasma clearance
C_{max}	maximum observed plasma concentration
CNS	central nervous system
CRF	case report form
CT	computerized tomographic scanning
CTFC	corrected TIMI Frame Count
DSMB	Data Safety Monitoring Board
ECG	electrocardiogram
ERC	Event Review Committee
EU	European Union
FDP	fibrinogen degradation products
GUSTO I trial	Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries. The GUSTO trial was designed to test the open artery hypothesis (if a given regimen produces a better patency rate and achieves reperfusion more rapidly, then mortality should also be reduced). It was the intent of GUSTO to incorporate the most aggressive thrombolytic regimens previously used that had also been shown to be safe and effective. The trial was designed to randomize sufficient subjects (> 41,000) to be able to detect a 1% difference in mortality between the various treatment groups.
ICH	intracranial hemorrhage
INR	international normalized ratio
IRA	infarct-related artery
ITT	intent-to-treat
IV	intravenous
LAD	left anterior descending (coronary artery)
LBW	lean body weight
LBBB	left bundle branch block
LRP	low-density lipoprotein receptor-related protein
LV	left ventricle; left ventricular
MI	myocardial infarction
MRI	magnetic resonance imaging
MRT	mean residence time in the plasma
PAP	plasmin-α_2-antiplasmin complex
PAI-1	plasminogen activator inhibitor type 1
PT	prothrombin time
PTCA	percutaneous transluminal coronary angioplasty
RR	relative risk
rt-PA	recombinant tissue-type plasminogen activator
SRP	Stroke Review Panel
$t_{1/2}$ initial	initial phase half-life in plasma
$t_{1/2}$ terminal	terminal phase half-life in plasma
TIA	transient ischemic attack
TIMI	Thrombolysis in Myocardial Infarction
TIMI trials	The Thrombolysis in Myocardial Infarction (TIMI) trials are a series of studies that began in 1984, initially under the sponsorship of the National Heart, Lung, and Blood Institute (NHLBI). These trials have examined a number of thrombolytic and antithrombotic regimens in acute myocardial infarction and unstable angina pectoris.
t-PA	tissue-type plasminogen activator
t-PA accelerated (Neuhaus) regimen	
> 67 kg:	100 mg administered over 90 minutes: 15 mg initial IV bolus, then 50 mg infused over

	30 minutes, followed by 35 mg infused over the next 60 minutes
≤ 67 kg:	15 mg initial IV bolus, then 0.75 mg/kg administered over 30 minutes and not exceeding 50 mg, followed by 0.50 mg/kg administered over the next 60 minutes and not exceeding 35 mg
t-PA ® standard regimen	
≥ 65 kg:	100 mg administered over 3 hours: 10 mg initial IV bolus, then 50 mg infused over 1 hour, followed by 20 mg/hr infused over the next 2 hours
< 65 kg:	1.25 mg/kg administered over 3 hours
V_1	initial volume of distribution
VSD	ventricular septal defect
V_{ss}	volume of distribution at steady-state
VT	ventricular tachycardia

Introduction

Acute Myocardial Infarction (AMI)

Despite major advances in prevention and treatment, acute myocardial infarction (AMI) remains the leading cause of death of both men and women in the U.S. The annual incidence of AMI is estimated to be 1.5 million in this country, with death in more than 500,000. The pathophysiology of AMI involves an underlying substrate of atherosclerotic coronary artery disease with superimposed plaque fissuring or rupture, leading to catastrophic coronary thrombosis and occlusion.

The most important therapeutic goal currently in the management of AMI is the prompt, complete and sustained restoration of antegrade perfusion following coronary occlusion. The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries-I (GUSTO-I) angiographic substudy suggested a relation between 90-minute patency of the infarct-related artery (IRA) with mortality. Regardless of the thrombolytic agent used, an occluded IRA (i.e., TIMI grade 0 or 1 flow) at 90 minutes has been associated with a 30-day mortality rate of ~8.9%, whereas the mortality rate with restoration of "normal" perfusion (TIMI grade 3 flow) is ~ 4.0%. Patients with "partial" perfusion (TIMI grade 2 flow) have an intermediate mortality rate. Similarly, higher TIMI flow grades have been associated with preservation of left ventricular (LV) function at 5 – 7 days.

Reperfusion in AMI can be achieved through pharmacologic means (i.e., use of thrombolytic agents), or mechanical means (primary percutaneous transluminal coronary angioplasty, PTCA). Though there has been rapidly increasing enthusiasm and use of primary angioplasty in the field, considerable use of thrombolytic agents remains, particularly at sites where cardiac catheterization laboratories are not available on a 24-hour basis.

Thrombolytic agents currently licensed for the treatment of AMI in the US include streptokinase, tissue plasminogen activator, anistreplase and reteplase. Their AMI indication statements are summarized as follows:

- Streptase (streptokinase) is indicated for use in the management of acute myocardial infarction (AMI) in adults, for the lysis of intracoronary thrombi, the improvement of ventricular function, and the reduction of mortality associated with AMI, when administered by either the intravenous or the intracoronary route, as well as for the reduction of infarct size and congestive heart failure associated with AMI when administered by the intravenous route…"

- Activase (rt-PA) is indicated for use in the management of acute myocardial infarction in adults for the improvement of ventricular function following AMI, the reduction of the incidence of congestive heart failure, and the reduction of mortality associated with AMI…."
- Eminase (anistreplase) is indicated for use in the management of acute myocardial infarction (AMI) in adults, for the lysis of thrombi obstructing coronary arteries, the reduction of infarct size, the improvement of ventricular function following AMI, and the reduction of mortality associated with AMI…."
- Retavase (reteplase) is indicated for use in the management of acute myocardial infarction (AMI) in adults for the improvement of ventricular function following AMI, the reduction of the incidence of congestive heart failure and the reduction of mortality associated with AMI…."

The proposed TNKase indication includes only a mortality reduction claim:

> **"TNKase is indicated for use in the reduction of mortality associated with acute myocardial infarction."**

Of note, left ventricular function was not systematically assessed in the phase 2 or 3 studies.

Overview of TNK Clinical Studies

Phase 1 Studies

TIMI 10A (N0647g)

Thrombolysis in Myocardial Infarction 10A (TIMI 10A, N0647g) was a Phase 1, open-label, dose-escalating (5 – 50 mg), angiographic pilot trial to assess the pharmacokinetics, safety and angiographic patency rates after single-dose IV TNK in AMI. Eligible subjects were < 70 years old, with symptom duration < 12 hours and ECG evidence of AMI or new left bundle branch block (LBBB), without contraindications to thrombolytic therapy. A total of 113 subjects received TNK in the study. Relative to historical experience with wild-type t-PA, TNK demonstrated decreased plasma clearance (mean 151 mL/min versus 572 mL/min for t-PA) and roughly a 5-fold prolongation in initial half-life (mean 17 min versus 3.5 min for t-PA). TIMI grade 3 flow was achieved in 57 – 65% of subjects at doses of 30 mg and above, with TIMI grade 2 or 3 flow in 81 – 88% subjects. No subjects experienced stroke or ICH. The 50-mg dose of TNK was associated with a 3% reduction in fibrinogen consumption and a 13% reduction in plasminogen levels, whereas the corresponding historical decreases for t-PA were roughly 50% (both fibrinogen and plasminogen levels). Serious hemorrhagic events were observed in 6 of the 113 subjects. Of these, 4 occurred at vascular access sites, and were not unexpected in light of the invasive procedures used in the angiographic protocol. There were four deaths through 30 days, all from causes associated with AMI. Adverse events were consistent with those events known to occur in the AMI patient population. Based on these data, TNK doses of 30 and 50 mg were selected for the phase 2 studies

Phase 2 Studies

The Phase 1 TIMI 10A study was followed by two phase 2 studies: TIMI 10B (N0660g) and ASSENT I (N0683g). These were randomized, multicenter, international trials for subjects with AMI presenting within 12 hours of symptom onset. Both were initially designed to assess the 30 and 50 mg doses of TNK. TIMI 10B was a medium-sized study (856 subjects) designed primarily to assess angiographic patency, whereas ASSENT I was a larger, 3235-subject study

designed to evaluate rates of ICH, stroke, death and serious bleeding complications. The two studies were conducted with a common Data and Safety and Monitoring Board (DSMB).

TIMI 10B (N0660g)

TIMI 10B was a randomized, open-label multicenter, international, angiographic trial of TNK compared with accelerated t-PA in subjects with AMI presenting within 12 hours of symptom onset. The study was initiated in March 1996, and enrolled 880 subjects over one year. The primary objective was to determine the patency rates (as TIMI grade 3 flow and TIMI frame counts) in the IRA 90 minutes after TNK (30 or 50 mg) or accelerated t-PA. Patency of the IRA was assessed arteriographically at 60, 75 and 90 minutes, with evaluations by a Core Angiography Laboratory, blinded to treatment assignment.

At interim analysis, there were 3 instances of ICH among 78 subjects who received the 50 mg TNK dose, leading the DSMB to recommend replacement of the 50 mg dose with a 40 mg dose in September 1996. The DSMB also recommended a decrease in the initial heparin dose for subjects ≤ 67 kg, earlier assessment of aPTT for heparin dose adjustments, more restricted use of heparin for rescue angioplasty and catheterization, a decrease in the maximum age to 80, and prohibition of the use of abciximab 96 hours prior to (as an exclusion criterion) and 96 hours after study entry.

Efficacy-evaluable data were obtained in 837 of 880 subjects enrolled. TNK doses of 40 and 50 mg were associated with TIMI grade 3 flow rates similar to those of subjects treated with accelerated t-PA, and the effects of TNK on angiographic patency appeared to be dose-related. The results of exploratory analyses suggested an association between log[TNK dose/weight] and TIMI grade flow, providing some of the rationale for use of a weight-standardized TNK dose in the subsequent Phase 3 trial.

Data from the subset of roughly half of the subjects in whom coronary arteriography was obtained at 60 and 75 minutes suggested that bolus administration of 40 or 50 mg TNK was as rapid in reestablishing antegrade perfusion as accelerated t-PA.

Rates of mortality and stroke in the TNK groups were generally comparable to those observed in the t-PA group, as well as those observed in previous angiographic trials of thrombolytic agents in AMI.

ASSENT I (N0683g)

ASSENT I, initiated in June 1996, was an uncontrolled 3235-subject study, originally designed to evaluate the safety of fixed 30 and 50 mg doses of TNK in patients with AMI presenting within 12 hours of symptom onset. The protocol was altered (along with TIMI 10B) in September 1996 because of concern regarding apparent excess ICH in the 50 mg TNK dose group in TIMI 10B. As in TIMI 10B, the 50 mg TNK dose was replaced with a 40 mg dose, heparin administration was decreased for subjects ≤ 67 kg and supplementary heparin administration was curtailed for diagnostic cardiac catheterization and rescue angioplasty. In addition, use of abciximab was prohibited in the 96 hours after randomization. The changes were made only 3 – 4 months after initiation, during enrollment “ramp up,” such that relatively few subjects (n=73) were randomized to the 50 mg TNK group, and only 10% of the subjects ultimately enrolled in the 30 mg group were treated before the protocol changes took effect. In essence, therefore, the study compared 30 mg TNK (n=1705) to 40 mg TNK (n=1457), with ~90% of the subjects in the 30 mg group and the entire 40 mg group randomized concurrently.

Thirty-day mortality rates were 6.9%, 6.0% and 4.1% for the 30, 40 and 50 mg dose groups, respectively. The respective reinfarction rates were 8.2%, 5.9% and 5.5%. ASSENT I (as well as TIMI 10A and TIMI 10B) enrolled subjects up to 12 hours after symptom onset. For subjects treated within 6 hours of symptom onset, mortality rates were 5.8%, 5.6% and 3.5% for the 30, 40 and 50 mg dose groups, respectively. For subjects treated after 6 hours of symptom onset, the mortality rates for these groups were 10.8%, 8.5% and 6.3%, respectively.

Respective rates of ICH were 0.9%, 0.6% and 0% for the 30, 40 and 50 mg dose groups. There was no apparent relation between ICH rates and either dose or dose/weight, although the numbers of ICH events were limited, and the analyses were confounded by a disproportionately high rate of missing weight data for subjects who experienced ICH.

Serious bleeding events occurred more frequently and were less obfuscated by missing data. There was a direct albeit somewhat weak relation between serious bleeding events and dose/weight. Taken into consideration with TIMI 10B data showing increased patency with increased log[dose/weight], these data provided support for a weight-adjusted TNK dosing paradigm for the phase 3 trial.

Phase 3 Study

ASSENT II (N0747g)

ASSENT II was the pivotal phase 3 trial, a randomized, double-blind, double-dummy, parallel-group, international trial of a single IV bolus of TNK versus accelerated t-PA in AMI. With a primary endpoint of 30-day mortality, the study was designed to test the hypothesis that weight-adjusted bolus administration of TNK was therapeutically comparable to t-PA with respect to mortality for the treatment of AMI. The study was conducted by Boehringer Ingelheim and Genentech, Inc. between October 1997 and December 1998, and encompassed 17005 subjects at 1022 sites.

The 30-day mortality rates (non-parametric adjusted rates) were 6.18% for TNK and 6.15% for t-PA, with the relative risk [RR] of TNK over t-PA of 1.004. The upper limit of the one-sided 95% CI for the RR was 1.104. Secondary and exploratory analyses of unadjusted rates and rates adjusted with logistic regression were consistent with the non-parametric adjusted rate. Upon exploratory subgroup analyses, the only potentially meaningful difference apparent between TNK and t-PA was observed in subjects treated 4 – 6 hours after symptom onset. In this subset of subjects, there was a trend towards lower mortality in TNK-treated subjects, although this association did not persist in multivariate analyses that included other independent predictors of mortality. Otherwise, no important disparities were identified in any subgroup with respect to mortality. Overall, total strokes and ICH were similar in frequency between the treatment groups; however, in subjects of African descent, there was an apparent trend towards excess non-hemorrhagic strokes in the TNK group. Secondary endpoints included a composite rate of death or non-fatal stroke at 30 days, rates of in-hospital myocardial reinfarction, pulmonary edema, cardiogenic shock and in-hospital invasive cardiac procedures. There were no statistically significant differences between groups with respect to these outcomes. There appeared to be fewer bleeding events and a lower incidence of anaphylaxis in TNK-treated subjects.

The individual studies are summarized in detail, below.

Protocol N0647g – TIMI 10A

Thrombolysis in Myocardial Infarction 10A (TIMI 10A, N0647g) was a Phase 1, open-label, dose-ranging (5 – 50 mg), angiographic pilot trial to assess the pharmacokinetics, safety and angiographic patency rates after single-dose bolus IV TNK in AMI. The study was co-sponsored by Boehringer Ingelheim/ and Genentech. Eligible subjects were < 70 years old, with symptom duration < 12 hours and ECG evidence of AMI or new left bundle branch block (LBBB), without contraindications to thrombolytic therapy.

Table 1: TIMI 10A 90-Minute Patency Rates

		Patency (% of Subjects)	
TNK dose	n	TIMI Grade 3	TIMI Grade 2 or 3
5 mg	5	20	80
7.5 mg	5	60	100
10 mg	5	40	80
15 mg	6	17	83
20 mg	7	29	71
30 mg	41	59	88
40 mg	21	57	81
50 mg	22	65	86
Overall	112		

A total of 113 subjects were enrolled at 18 hospitals between January and November, 1995. All received TNK in the study. Mean age was 54 years, 84% were male, and 38% had anterior MI. Relative to historical experience with wild-type t-PA, TNK demonstrated decreased plasma clearance (mean 151 mL/min versus 572 mL/min for t-PA) and roughly a 5-fold prolongation in initial half-life (mean 17 min versus 3.5 min for t-PA). The 50 mg TNK dose was associated with a 3% reduction in fibrinogen consumption and a 13% reduction in plasminogen levels, whereas the corresponding historical decreases for t-PA were roughly 50% (both fibrinogen and plasminogen levels).

TIMI grade 3 flow was achieved in 57 – 65% of subjects at doses of 30 mg and above, with TIMI grade 2 or 3 flow in 81 – 88% subjects. There was a trend towards slightly higher patency rates with the 50 mg dose compared to the other doses. No subjects experienced stroke or ICH. Serious hemorrhagic events were observed in 6 of the 113 subjects. Of these, 4 occurred at vascular access sites. There were four deaths through 30 days, all from causes associated with AMI. Other adverse events were consistent with those events known to occur in the AMI patient population. There were no reports of anaphylaxis during the study. At baseline, 11 subjects tested positive for antibodies to TNK, and 3 additional subjects were positive at hospital discharge, suggesting cross-reactivity and/or limited specificity of the assay. There were 92 subjects in whom antibody response was assessed at Day 30 (actual range: day 23 to 76), and none tested positive for anti-TNK antibodies.

Based on historical data from the GUSTO I angiographic substudy, 30 mg TNK appeared likely to have approximately the same enzymatic effect as 100 mg accelerated t-PA (59% TIMI grade 3 flow, 95% CI 42 - 73%). Because the 50 mg dose appeared to be more effective than 30 or 40 mg, and because there were no strokes or ICH in this group (though experience was limited to 22 subjects), TIMI 10B was planned comparing t-PA to both 30 and 50 mg doses of TNK. (ASSENT I was planned to assess the 30 and 50 mg doses of TNK.)

Protocol N0660g – TIMI 10B

Title: A Phase II, Randomized, Open-Label, Multicenter, International, Angiographic Trial of the Efficacy of TNK Compared With Accelerated T-PA Alteplase rt-PA in Acute Myocardial Infarction (TIMI 10B Study)
Study Period: March 1996 – April 1997
Funding: Boehringer Ingelheim GbmH, Germany; Genentech, Inc., USA

Centers: Seventy-four sites in the US, Germany, France, Belgium, Canada, Netherlands, and UK

The phase 2 protocol was submitted to CBER February 13, 1996 as amendment 24 to IND 5880. CBER had no major concerns regarding the study design.

Objectives

The stated primary study objective was to determine the percentage of subjects with TIMI grade 3 flow in the IRA 90 min after initiation of treatment with bolus TNK (initially 30 and 50 mg; after amendment, 30 and 40 mg) compared with accelerated t-PA. Secondary objectives included:

- assessment of IRA patency (TIMI grade flow and TIMI frame count) at 60, 75 and 90 min
- assessment of safety and clinical efficacy of TNK
- evaluation of effects of TNK on coagulation factors and fibrinogenolysis
- evaluation of the formation of antibodies against TNK
- assessment of TNK pharmacokinetics (selected clinical sites)
- assessment of angiographic reocclusion rate at 18–36 hours after treatment (at selected clinical sites)

Study Design

Overview

This was a Phase 2, randomized, open-label, multicenter, international, angiographic trial of TNK compared with accelerated t-PA in subjects with AMI, presenting within 12 hours of symptom onset. As the protocol was originally written, subjects were randomized (1:1:1) to receive 30 or 50 mg TNK given as an IV bolus over 5–10 seconds, or accelerated weight-adjusted t-PA. Aspirin and IV heparin were given at study entry. Because of concerns of increased bleeding events associated with administration of the 50 mg TNK dose, the 50 mg dose was replaced with a 40 mg dose approximately 40% of the way through the study. TIMI grade flow and TIMI frame count were assessed 90 minutes after the start of TNK or t-PA administration, and also at 60 and 75 minutes, when feasible.

Administrative Structure

Genentech had primary responsibility for the North American sites; Boehringer Ingelheim had primary responsibility for non-North American sites. The trial was performed concurrently with ASSENT I (Genentech N0683g; Boehringer Ingelheim 1123.3), with common Operations Committee and DSMB, such that interim efficacy and safety analyses from either study could prompt changes in both.

Operations Committee

Operations Committee members included the study chairs of this trial and ASSENT I, and representatives of the sponsors. The Operations Committee reviewed unblinded safety data from both clinical trials, with particular attention to ICH. Based on their review of the safety data, the Committee could initiate an unscheduled review by the DSMB.

Data and Safety Monitoring Board

A DSMB, comprised of 4 independent cardiologists and an independent biostatistician, monitored both TIMI 10B and ASSENT I. The DSMB conducted scheduled, unblinded interim

safety and efficacy analyses when 150 evaluable subjects were enrolled (50 subjects/treatment group). Safety data from ASSENT I were to be included in the interim analysis. The DSMB was to conduct additional unblinded analyses of safety and efficacy at the request of the Operations Committee. The DSMB was empowered to recommend to the Sponsors' Data Review Board that a dose level of TNK be terminated and/or changed, or that the Phase 2 trials be terminated. The Study Chair participated in the DSMB's discussions of the unblinded safety and efficacy data.

Event Review Committee

An Event Review Committee (ERC), consisting of independent cardiologists and neurologists blinded to treatment group, reviewed documentation from stroke and death events. The ERC assessed the nature of strokes and classified each as hemorrhagic versus non-hemorrhagic; the ERC also assessed each death with regard to its relationship to the AMI.

Definitions of Terms

Intracranial Hemorrhage

Intracranial hemorrhage (ICH) was defined as stroke with focal collections of intracerebral blood observed on head computerized tomography (CT) scanning or magnetic resonance imaging (MRI), or on post-mortem examination, not thought to represent hemorrhagic conversion (subarachnoid and subdural hemorrhage included).

Stroke

Stroke was defined as the sudden onset of a focal neurologic deficit not resolving spontaneously within 24 hours; and not a temporary result of pharmacologic intervention(s), e.g., lidocaine, sedation, or analgesics.

Bleeding Classification

- Mild bleeding was defined as neither requiring transfusion nor causing hemodynamic compromise (i.e., subcutaneous bleeding, minor hematomas, etc.)
- Moderate bleeding was defined by a requirement for blood transfusion, in the absence of hemodynamic compromise requiring intervention.
- Severe/life-threatening bleeding was defined as that which causes hemodynamic compromise requiring intervention and transfusion. All ICH was considered severe/life threatening bleeding, regardless of transfusion or hemodynamic status.

Anaphylaxis

Anaphylaxis was defined as vascular collapse and shock (BP < 90 mmHg, unresponsive to IV fluids) believed to be allergic in origin, with or without antecedent respiratory distress, occurring within 30 minutes of initiation of study drug administration. Cutaneous manifestations could include pruritus, urticaria or angioedema.

Cardiogenic Shock

Cardiogenic shock was defined as a systolic BP (BPs) < 90 mmHg with IV vasopressors or < 80 mmHg without vasopressors, together with tissue hypoperfusion evidenced by oliguria, heart rate >100 beats/minute, decreased level of consciousness and cool/clammy skin.

TIMI grade flow

TIMI grade flow was classified as follows (standard classification):
Grade 0: no perfusion
Grade 1: penetration without perfusion
Grade 2: partial perfusion
Grade 3: complete perfusion

TIMI frame count

TIMI frame count was defined as the number of cine frames required for opacification of the distal IRA. The count was determined locally (using methods delineated in a videotape provided by the [redacted] and by the [redacted] reader. If the IRA was occluded (i.e., TIMI flow of 0 or 1), an imputed value of 100 was assigned. In subjects with a left anterior descending (LAD) IRA, the frame count value was divided by 1.7 to obtain the corrected TIMI frame count (CTFC).

Patient Population

Subjects with AMI who presented within 12 hours after symptom onset comprised the patient population.

Inclusion Criteria

- ischemic discomfort ≥ 30 minutes in duration
- ST segment elevation ≥ 0.1 mV in two contiguous ECG leads indicative of AMI
- ability to be randomized within 12 hours after AMI symptom onset
- age ≥ 18 and < 80 years

Exclusion Criteria

- MI treated with any thrombolytic agent within the preceding 4 days
- previous CABG surgery
- cardiogenic shock (e.g., BPs < 90 mmHg with IV vasopressors or < 80 mmHg without IV vasopressors)
- HTN ≡ BP > 180/110 mmHg (BPs > 180 mmHg and/or BPd > 110 mmHg)
- inability to undergo cardiac catheterization
- significant bleeding disorder within the past 6 months
- use of abciximab within 96 hours
- major surgery, biopsy of a parenchymal organ, or significant trauma within 3 months
- history of stroke or transient ischemic attack (TIA) or central nervous system (CNS) structural damage (i.e., neoplasm, aneurysm, intracranial surgery)
- oral anticoagulation - international normalized ratio [INR] ≥ 1.4; prothrombin time [PT] ≥ 14 sec
- prolonged cardiopulmonary resuscitation (> 2 minutes) within 2 weeks
- noncompressible vascular puncture within 10 days
- pregnancy (positive urine pregnancy test) or lactation, parturition within the previous 30 days, or woman with childbearing potential not using adequate contraception
- other serious illness (e.g., active cancer, active infection)
- current cocaine abuse
- previous treatment with TNK
- new LBBB only (not eligible because of the difficulty of determining the IRA)

Treatment

Material Source

TNK and t-PA were provided as sterile, lyophilized powders for reconstitution with Sterile Water for Injection, USP (or EP for non–North American sites). Each TNK vial contained 20 mg of TNK and the following excipients: arginine, phosphoric acid, and polysorbate 20. The TNK vials were to be reconstituted in 4 mL of Sterile Water for Injection (SWFI), resulting in a TNK concentration of 5 mg/mL. Each t-PA vial contained 50 or 100 mg of t-PA Alteplase and was to be reconstituted with SWFI, 50 or 100 mL, respectively, for a concentration of 1 mg/mL. The t-PA contained the following excipients: arginine, phosphoric acid, and polysorbate 80. The xxxxx brand of t-PA was not used in the US or in Europe.

Randomization

Randomization was to be blocked by study site, based on a SAS-generated code. Treatment assignment was made through a call to a centralized interactive voice response system for a drug kit number. Subjects were considered randomized upon verbal assignment of kit number.

Blinding

Blinded angiographic interpretation was performed at the at the [redacted] at the [redacted]

Dose and Administration

TNK was administered as an IV bolus injection over 5–10 seconds. The originally planned doses of TNK were 30 and 50 mg. After the September 19, 1996 protocol amendment, the planned doses were 30 or 40 mg.

t-PA was administered as an accelerated 90-minute infusion regimen. For subjects weighing >67 kg, 100 mg was to be administered: 15 mg as an IV bolus, followed by a 50 mg infusion over 30 minutes, and then a 35 mg infusion over the next 60 minutes. Subjects ≤ 67 kg were to receive a 15 mg initial IV bolus, then 0.75 mg/kg administered over 30 minutes (not to exceed 50 mg), followed by 0.50 mg/kg administered over the next 60 minutes (not to exceed 35 mg).

Concomitant Medications

Specially Directed:

- Aspirin, 150-325 mg PO upon study entry, then 150-325 mg qd. Alternatively, 100-250 mg aspirin IV was acceptable where approved by local regulatory authorities. For subjects who had taken aspirin within 24 hours prior to randomization, aspirin was begun the following day.
- Heparin, 4000 units IV bolus, then 800 units/h infusion (subjects ≤ 67 kg); or 5000 units IV bolus, then 100 units/hour (subjects > 67 kg) for 48–72 hours. The bolus was to be administered as soon as possible after baseline aPTT was obtained. aPTT was to be performed at baseline, 6, 12, 24 and 48 hours after the start of study agent, and daily thereafter while on IV heparin. Infusion rate was to be adjusted to maintain aPTT at 55–80 seconds; aPTT was to be assessed 6 hours after adjustments in infusion rate. After the 9/96 protocol amendment, no additional heparin was to be given during diagnostic catheterizations while subjects were on IV heparin. Additional, large heparin doses were to be avoided if rescue angioplasty was performed. If given, heparin was administered in increments no larger than 2500 units.

Prohibited:

- abciximab during the first 96 hours after randomization (after 9/96 protocol amendment)
- investigational medications or investigational devices through the 30-day follow-up period

Discretionary:

- β-adrenergic blocking agents
- calcium antagonists

Interventions

- PTCA permitted after the 90–minute angiogram, or before 90 minutes for rapid and progressive hemodynamic deterioration.
- stenting permitted after the 90–minute angiogram
- CABG permitted after the 90–minute angiogram

Monitoring

Scheduled Evaluations

Scheduled evaluations are summarized in Table 2.

Coronary Arteriography

All angiograms were to be performed using standard operating procedures typically used in angiographic studies for assessment of TIMI flow rates and frame counts. Coronary arteriography was to be performed at 90 minutes, and at 60 and 75 minutes, when feasible. To limit the potential for contrast-mediated reperfusion, only one injection of the IRA was to be performed at 60 and 75 minutes. For 90-minute arteriography, determination of the TIMI grade

Table 2: TIMI 10B Study Flowchart

	Baseline	2 min	30 min	60 min	75 min	90 min	2 hr	3 hr	6 hr	8 hr	12 hr	16 hr	24 hr	48 hr	discharge	30 Days
History/physical/ ECG	X														X	
Cineangiography: TIMI grade flow and frame count [1]				X[1]	X[1]	X										
Urinalysis	X												X			
Hematology/ Chemistry [2]	X												X		X	
PT	X															
aPTT	X								X		X		X	X		
Antibodies [3]	X															X
CK/CK-MB [4]	X									X		X				
troponin I, myoglobin, and CK-MB	X			X		X		X			X		X	X		
PK	X	X	X	X		X	X	X	X							
fibrinogen, FDPs, or D-dimer	X			X				X	X[5]							
clinical status, adverse events, and clinical outcome																X

[1] additional arteriograms @ at 60 and 75 min, if possible. At selected sites, additional arteriograms at 18–36 hours in subjects with TIMI grade 3 flow at 90 minutes to assess reocclusion.

[2] performed any time within initial 24-48 hours: hemoglobin, hematocrit, WBC, platelets, Na, K, Cl, BUN, creatinine, glucose, SGPT (ALT), SGOT(AST), and LDH.

[3] performed only on subjects receiving TNK. A 90-day follow up was included for subjects with positive antibodies at Day 30.

[4] Also obtained for signs or symptoms suggestive of recurrent MI.

[5] selected clinical sites

flow was to be based on the initial injection of the IRA. Additional views of the IRA could be obtained to provide adequate visualization of the culprit lesion if deemed necessary. Left ventriculography was optional. At selected sites, an additional catheterization was to be performed 18–36 hours after the start of treatment for assessment of reocclusion.

Clinical Assessment

Suspected ICH

Subjects with a change in neurological function at any time during the study were to undergo immediate cranial CT scanning (preferred) or MRI (also acceptable).

30-Day Follow-Up

Clinical status, adverse events and clinical outcome was to be determined and reported in the CRFs.

90-Day Follow-Up

Subjects testing positive for anti-TNK antibodies at Day 30 were to undergo repeat assessment at 90 ±10 days.

Response Variables

Primary Endpoint

The prospectively-defined primary efficacy endpoint was the proportion of TIMI grade 3 flow in the IRA 90 minutes after initiation of study agent.

Secondary Endpoints

- TIMI grade 2 or 3 flow in the IRA – 90 minutes after start of study drug administration
- TIMI frame count in the IRA – 60, 75 and 90 minutes after start of study drug administration (as continuous variables and as % of subjects with corrected TIMI frame count (CTFC) <40)
- TIMI grade 3 flow and TIMI grade 2 or 3 flow in the IRA 60 and 75 minutes after start of study drug administration
- TNK pharmacokinetics
- Safety as determined by the incidences of death, recurrent MI, total strokes (hemorrhagic and non-hemorrhagic), ICH, serious/life threatening bleeding complications, coronary revascularization, pulmonary edema, cardiogenic shock, anaphylaxis and other AEs
- Incidence of antibody formation against TNK

Statistical Analysis Plan

Subjects with an evaluable angiogram were to be included in the efficacy analyses. Subjects with non-evaluable angiograms were to be replaced. Missing values were not interpolated. Subjects who received study agent were to be included in the safety analyses. Statistical comparisons were to be 2-tailed with α=0.05. Pairwise comparisons between groups were to be made without adjustment for multiplicity.

Baseline demographic and clinical data were to be assessed for comparability between treatment groups, with appropriate adjustments for any potential confounders and modifiers. Between-group analyses were to be performed using a one-way analysis of variance (ANOVA)

and a two-sample t-test for continuous variables. For discrete variables, Pearson chi-squared test was to be performed for between-group analyses when expected cell counts were > 5, otherwise, Fisher's exact test was to be used. The Mantel-Haenszel chi-squared test was to be used for ordinal variables. Exploratory analyses of patency by time to treatment and IRA were planned. Correlation between core laboratory and investigator readings of TIMI grade flow were to be analyzed by a weighted κ value. Safety was to be assessed primarily through the summary of bleeding, other adverse events and laboratory test results, including changes in coagulation parameters. Adverse events were to be tabulated by body system and treatment.

Interim Analyses

An interim efficacy analysis was prospectively planned when [redacted] readings of 90-minute patency were available for at least 50 subjects in each of the 3 initial treatment groups. The primary purpose of the interim analyses was to evaluate TNK efficacy as measured by patency. There were stopping rules for low patency rates. (As a result of the 9/96 protocol amendment, an interim efficacy analysis was added for the 25- and 40-mg TNK doses.)

TIMI 10B Study Results

The first subject was enrolled in March 1996. Enrollment was completed in one year, with a study duration of 13 months (including 30-day follow-up).

Protocol Amendment

By August 1996 there were three ICHs among the 78 subjects in the 50 mg TNK dose cohort. (There with no ICHs among the 73 subjects treated with 50 mg in ASSENT I, which had begun enrolling subjects in June 1996). The Operations Committee conferred with the Sponsors' Data Review Board and the DSMB Chair, and although the ICH rate in the 50 mg TNK group did not meet the pre-specified bound of the lower 95% CI $\geq$ 1%, it was decided to suspend the 50 mg dose in both trials. Plans were made to introduce 25 and 40 mg TNK doses; however, after a subsequent review of interim safety and efficacy data in October 1996 by the Study Chair and DSMB Chair, it was decided to introduce only the 40-mg dose.

A single protocol amendment, dated September 19, 1996, implemented the following changes:

- the 50-mg TNK dose was discontinued
- A TNK dose of 40 mg was added
- age < 80 years was added as an inclusion criterion
- concomitant medications that might influence the potential risk of major hemorrhage were restricted, including:
  - restriction of additional heparin use during catheterization and rescue angioplasty to increments of 2500 units, with a target activated clotting time (ACT) of 300 seconds
  - reduction of the initial heparin bolus for subjects weighing $\leq$ 67 kg; titration of heparin infusion to the aPTT was to begin at 6 hours
  - prohibition of the use of abciximab within 96 hours before and after randomization

Patient Enrollment, Treatment Assignment and Compliance

A total of 880 subjects were enrolled in the study. The first subject was enrolled at the Sarasota Florida site on March 7, 1996. The rate of enrollment increased throughout the first four study months, reaching a stable plateau of ~100 subjects enrolled per month. Enrollment was completed within a 12-month period. Three hundred sixty-two (362) subjects were enrolled prior to the September 1996 protocol amendment; 518 subjects were enrolled after the amendment,

accounting for disparities in numbers of subjects in each treatment arm. Subjects were enrolled from seven countries, with 56% of subjects enrolled at US sites. Enrollment by country is summarized as follows: the US (n=472), France (n=93), Belgium (n=83), UK (n=67), Canada (n=66), Germany (n=35) and the Netherlands (n=21). *Ninety-seven percent of subjects (851 subjects) received the assigned treatment and dose.*

The breakdown of the 29 subjects who did not receive the planned treatment is as follows:

3 were treated but not randomized
26 were randomized but received the wrong treatment or no treatment as follows:
2 received wrong study drug/dose (1 TNK instead of t-PA; 1 incorrect TNK dose)
11 received a non-study thrombolytic agent
13 were not treated as follows:
3 were taken directly to the cath lab (2 underwent primary PTCA)
8 had resolution of symptoms and/or ECG changes
2 died before the study drug could be administered

The safety-evaluable study population consisted of the 856 subjects who received a study agent and are denoted (above) with italics font. This included the 851 subjects treated as assigned, as well as the 3 subjects treated but not randomized and the 2 subjects who received the incorrect study agent and/or dose. Of the 856 subjects comprising the safety-evaluable cohort, 837 (98%) had an evaluable 90-minute angiogram and comprised the efficacy-evaluable cohort. The study populations are summarized in Table 3.
All but 4 TNK subjects were administered the correct dose (per the actual treatment kit provided in-hospital). Three subjects were to receive 40 mg but only received 30 mg; 1 subject was to receive 30 mg but actually received 40 mg.
Data were analyzed according to the treatment kit received (TNK versus t-PA), regardless of actual dose administered, with the exception of the PK analysis of TNK, where data were analyzed according to actual dose received.
Reviewer's Comment(s): Because local medical practices and technical factors may affect the performance of coronary arteriography upon which the TIMI flow and CTFC are based, maintenance of balance within sites is a laudable goal of an angiographic study. Although randomization was blocked by site, over half the sites (57%) enrolled fewer than 9 subjects, and balance was largely not achieved at these sites. Moreover, there were notable imbalances in enrollment at many of the sites where the numbers of subjects would have been adequate to achieve balance. These imbalances were an indirect result of the 9/96 protocol amendment. Specifically, there was a 3-month period between 8/19/96 and

Table 3: Safety and Efficacy Evaluable Subjects by Treatment Group

	TNK 30 mg	TNK 40 mg	TNK 50 mg	t-PA	total
Enrolled	316	157	82	325	880
Treated (safety evaluable)	308	154	78	316	856
Treated - had 90-minute angiogram (efficacy evaluable)	302	148	76	311	837

11/25/96 after the 50 mg group had been discontinued and before the 40 mg group had been initiated. During this period, ~300 subjects were randomized 1:1 between t-PA and 30 mg TNK. Once the 40 mg TNK dose was available in late November 1996, subjects were randomized between TNK 30 mg, TNK 40 mg, and t-PA in a ratio approximating 1:2:1 (for the study overall). Thus, balance or imbalance

between study groups at a given site was dependent on the time frame during which the site enrolled subjects. In light of the imbalances in treatment group allocation at larger sites, and the fact that the majority of sites had such limited enrollment that balance was largely not achieved, the study can not be considered to be well-balanced on the whole with respect to subject allocation to treatment group within sites.

Protocol Deviations

Eight subjects (0.91%) did not meet inclusion/ exclusion criteria and were known ineligible at study entry. Four were > 80 years-old, one failed to meet ECG ST segment criteria, one was randomized > 12 hours after symptom onset and two subjects were enrolled despite the presence of exclusion criteria (one with a previous CABG and history of stroke, TIA or CNS damage, another with blood pressure >180/110 mmHg). On the basis on information obtained through the follow-up period, 53 (6%) of the 880 subjects were found, retrospectively, not to have met study eligibility criteria.

Reviewer's Comment(s): The majority of violations were for factors that could negatively impact safety. In this category, violations in decreasing order of frequency were: BP > 180/110 (n=14), therapeutic anticoagulation (n=9), age > 80 (n=4), major surgery or trauma within 3 months (n=4), shock (n=1), and current cocaine abuse (n=1). Of concern with respect to the demonstration of efficacy are factors that would predict patency of the IRA at baseline (i.e., factors suggesting that a subject did not truly have an AMI – thus an insurance of "success"). In this category, there are only 3 subjects, all of whom failed to meet ECG criteria for entry. This negligible fraction of subjects is unlikely to importantly affect the study results. The presence of other exclusions has the effect of causing an apparent reduction in safety, because they tend to predispose subjects to adverse events.

Subject Follow-Up

Of the 856 subjects who received study drug, 778 (91%) completed the 30-day follow-up visit. Forty-three subjects (5%) died during the 30-day study period, 7 (0.8%) withdrew because of noncompliance with the study, 11 (1.3%) withdrew for other reasons and 17 (2%) were lost to follow-up.

Study Population: Baseline Characteristics

General

Table 4 provides a summary of baseline demographic characteristics and cardiovascular disease status by treatment group for the safety evaluable population (subjects who received study agent). Generally, baseline characteristics are typical of subjects in previous trials of thrombolytic agents, and there was excellent balance between treatment groups.

Reviewer's Comment(s): The time from hospital arrival to study agent initiation variable was affected by numerous subjects who developed AMI while hospitalized, as evidenced by the fact that they received study agent > 12 hours to several days after hospital arrival. Presumably, many of these subjects developed AMI while hospitalized for unstable angina. For this reason, the utility of the time from hospital arrival to study agent initiation is of questionable utility in this study.

Table 4: **Demographic Characteristics and Baseline Disease Status (TIMI 10B)**

Characteristic	TNK 30 mg	TNK 40 mg	TNK 50 mg	t-PA	total
N	308	154	78	316	856
age [years]					
median	60.0	58.0	60.0	59.0	59.0
mean (SD)	60.1 (11.8)	58.6 (11.8)	60.3 (11.7)	59.5 (11.9)	59.6 (11.8)
range	33 - 87	34 - 85	35 - 93	28 - 86	28 - 93
number (%) male					
	238 (77.3)	107 (69.5)	58 (74.4)	248 (78.5)	651 (76.1)
race [n (%)]					
Caucasian	235 (76.3)	100 (64.9)	63 (80.8)	238 (75.3)	636 (74.3)
African descent	15 (4.9)	9 (5.8)	3 (3.8)	15 (4.7)	42 (4.9)
Asian	10 (3.2)	5 (3.2)	2 (2.6)	11 (3.5)	28 (3.3)
Hispanic	18 (5.8)	13 (8.4)	3 (3.8)	20 (6.3)	54 (6.3)
other	3 (1.0)	0 (0.0)	0 (0.0)	1 (0.3)	4 (0.5)
unable to provide	27 (8.8)	27 (17.5)	7 (9.0)	31 (9.8)	92 (10.7)
subject mass [kg]					
median	78.4	77.3	80.0	77.6	78.0
mean (SD)	79.6 (16.4)	79.4 (17.4)	79.6 (16.7)	78.6 (16.0)	79.2 (16.4)
range	44.2 - 150	40.5 - 188.5	40.5 - 127.8	44.0 - 157.8	40.5 - 188.5
≤ 67 [n (%)]	68 (22.1)	29 (18.8)	18 (23.1)	68 (21.5)	183 (21.4)
> 67 [n (%)]	240 (77.9)	125 (81.2)	60 (76.9)	248 (78.5)	673 (78.6)
infarct location [n (%)]					
anterior	122 (39.6)	64 (41.6)	29 (37.2)	116 (37.1)	331 (38.8)
inferior	181 (58.8)	87 (56.5)	45 (57.7)	186 (59.4)	499 (58.5)
lateral	5 (1.6)	3 (1.9)	4 (5.1)	11 (3.5)	23 (2.7)
posterior	0	0	0	0	0
missing	0	0	0	3 (0.9)	3 (0.4)
infarct related artery [n (%)]					
LAD	110 (36.1)	56 (37.6)	29 (38.2)	108 (34.5)	303 (35.9)
LCX	40 (13.1)	18 (12.1)	10 (13.2)	48 (15.3)	116 (13.8)
RCA	155 (50.8)	75 (50.3)	37 (48.7)	157 (50.2)	424 (50.3)
previous medical history [n (%)]					
previous MI	49 (15.9)	23 (14.9)	12 (15.4)	46 (14.6)	130 (15.2)
CAD/angina	77 (25.0)	42 (27.3)	19 (24.4)	86 (27.2)	224 (26.2)
previous CHF	9 (2.9)	4 (2.6)	3 (3.8)	6 (1.9)	22 (2.6)
HTN	113 (36.7)	63 (40.9)	33 (42.3)	131 (41.5)	340 (39.7)
PTCA (previous)	23 (7.5)	13 (8.4)	8 (10.3)	28 (8.9)	72 (8.4)
↑ cholesterol	107 (34.7)	62 (40.3)	19 (24.4)	121 (38.3)	309 (36.1)
diabetes	47 (15.3)	24 (15.6)	14 (17.9)	48 (15.2)	133 (15.5)
current smoker	154 (50.0)	78 (50.6)	33 (42.3)	138 (43.7)	403 (47.1)
time from symptom onset to study drug [hours]					
median	2.8	2.9	2.9	2.9	2.9
mean (SD)	3.5 (2.3)	3.4 (2.1)	3.6 (2.4)	3.6 (2.2)	3.5 (2.2)
range	0.6 - 12.3	0.5 - 11.9	1.0 - 11.9	0.7 - 11.5	0.5 - 12.3
0 - 3	171 (55.9)	84 (54.9)	42 (53.8)	172 (54.4)	469 (55.0)
> 3 - 6	94 (30.7)	52 (34.0)	26 (33.3)	104 (32.9)	276 (32.4)
> 6 - 12	40 (13.1)	17 (11.1)	10 (12.8)	40 (12.7)	107 (12.5)
> 12	1 (0.3)	0	0	0	1 (0.1)

Concomitant and excluded medications

Concomitant medication use was similar across treatment groups. Overall, 93.3% of subjects received aspirin, and 97.2% and 99.6% of subjects received a heparin bolus and infusion, respectively. Twenty-three percent of subjects who received heparin received a bolus dose that was too high, based on the recommendations after the protocol amendment. Twenty-two percent of subjects received an excessive heparin infusion dose. For the study as a whole, IV nitrate and beta-blocker use were recorded commonly, in 83% and 77% of subjects, respectively, without significant differences between treatment groups. Use of ticlopidine was relatively common (17% overall). Abciximab use was proscribed and was uncommon, reported in 1.9% of subjects overall.

Table 5: Concomitant Medication Use – Day 0 and 1 (TIMI 10B)

	TNK 30 mg	t-PA 40 mg	TNK 50 mg	t-PA	overall
n	308	154	78	316	856
Aspirin	95.0%	94.6%	88.2%	92.3%	93.3%
Heparin					
Bolus (%)	97.7%	98.0%	100.0%	95.5%	97.2%
Infusion (%)	100.0%	99.3%	98.7%	99.7%	99.6%
IV nitrates (%)	82.8%	83.1%	82.9%	83.9%	83.3%
Oral/ topical nitrates (%)	14.9%	19.6%	28.9%	19.6%	18.8%
Beta blockers (%)	78.5%	81.8%	77.6%	72.0%	76.6%
ACE inhibitors (%)	27.2%	33.8%	34.2%	29.9%	30.0%
Calcium Antagonists (%)	17.9%	15.5%	18.4%	18.6%	17.8%
Ticlopidine (%)	17.5%	23.6%	11.8%	14.5%	17.0%
Abciximab (%)	2.3%	2.7%	3.9%	0.6%	1.9%
Re-administration of thrombolytics (%)	0.0%	0.0%	0.0%	0.0%	0.0%
Additional thrombolytics (%)	0.3%	0.0%	0.0%	0.3%	0.2%

Primary Efficacy Endpoint: TIMI Grade 3 Flow at 90 Minutes

The primary efficacy data are summarized in Table 6. TIMI Grade 3 flow tended to occur less frequently in the 30 mg TNK group; the only statistically significant pairwise comparison was between the 30 mg TNK group versus the t-PA group (p=0.035, not corrected for multiplicity of analyses).

Table 6: TIMI Grade 3 Flow at 90 Minutes: Efficacy Evaluable Cohort

	TNK			t-PA
	30 mg	40 mg	50 mg	100 mg
N	302	148	76	311
Number of subjects (%)	164 (54.3%)	93 (62.8%)	50 (65.8%)	195 (62.7%)
95% CI	(48.5%, 60%)	(54.5%, 70.6%)	(54%, 76.3%)	(57.1%, 68.1%)

Exploratory Subgroup Analyses on The Primary Angiographic Patency Endpoint

Subgroup Analyses

CBER conducted exploratory analyses to examine the data for indications of patient subsets that may not have derived benefit from TNK administration, while still incurring adverse effects. Such patients would have an unfavorable risk to benefit comparison. Table 7 summarizes CBER's exploratory analyses of 90-minute TIMI 3 flow rates for subgroups divided by age, gender, weight, "symptom-to-needle" time, IRA, and time of study entry (before or after the 9/19/96 protocol amendment). With the division of treatment groups into subgroups, the numbers of subjects per treatment arm/subgroup are somewhat limited. Nevertheless, 90-minute TIMI Grade 3 flow rates appear similar across age, gender, weight and IRA subgroups of each treatment group. For all groups, TIMI Grade 3 flow rates appear to decrease with increasing "symptom-to-needle" time. Results are consistent between the two periods of study entry for the 30-mg TNK and t-PA groups (the only groups enrolling subjects in both study halves).

Timeliness of Catheterization

Because thrombolysis is a dynamic event, the timing of coronary arteriography could importantly affect TIMI flow results. CBER assessed the punctuality of arteriography for each group individually, and for the study as a whole. Timeliness was assessed by comparing coronary arteriography times with bolus times, and characterizing the difference by categories of: >15, 10–15, or 5–10 minutes early; ± 5 minutes of target time; 5–10, 10–15 or >15 minutes late. No important disparities were apparent between treatment groups with respect to punctuality. For the study as a whole, 78% of the 90–minute arteriograms were performed within 5 minutes of the target time, with 88% and 92% conducted within ±10 and ±15 minutes, respectively. Five percent (5%) were performed > 15 minutes from the target time, and 4% of subjects were missing a recorded time of catheterization.

Table 7: CBER's Exploratory Subgroup Analyses on TIMI Grade 3 Flow at 90 Minutes

	TNK 30 mg			TNK 40 mg			TNK 50 mg			t-PA		
	n	patent	%	n	patent	%	n	patent	%	n	patent	%
Overall	302	164	54.3%	148	93	62.8%	76	50	65.8%	311	195	62.7%
Age (years)												
≤ 65	194	107	55.2%	100	69	69.0%	50	32	64.0%	207	133	64.3%
65 - 75	79	41	51.9%	35	20	57.1%	17	12	70.6%	76	47	61.8%
> 75	29	16	55.2%	13	4	30.8%	9	6	66.7%	28	15	53.6%
Gender												
male	233	128	54.9%	103	63	61.2%	56	37	66.1%	244	151	61.9%
female	69	36	52.2%	45	30	66.7%	20	13	65.0%	67	44	65.7%
Weight												
≤ 67	67	41	61.2%	28	16	57.1%	18	15	83.3%	67	39	58.2%
> 67	235	123	52.3%	120	77	64.2%	58	35	60.3%	244	156	63.9%
"Symptom-to-Needle" time												
0 - 3 hours	168	101	60.1%	80	51	63.8%	42	27	64.3%	171	110	64.3%
3 - 6 hours	92	44	47.8%	50	32	64.0%	24	15	62.5%	101	62	61.4%
6 - 12 hours	39	17	43.6%	17	9	52.9%	10	8	80.0%	39	23	59.0%
> 12 hours	1	0	0.0%	0			0			0		
Infarct related artery												
LAD	109	46	42.2%	55	25	45.5%	29	15	51.7%	108	60	55.6%
LCx	40	16	40.0%	18	11	61.1%	10	9	90.0%	47	30	63.8%
RCA	153	102	66.7%	75	57	76.0%	37	26	70.3%	156	105	67.3%
Time of study entry relative to 9/19/96 amendment												
pre-	130	68	52.3%	0	0		75	49	65.3%	140	89	63.6%
post-	171	96	56.1%	148	93	62.8%	1	1	100%	171	106	62.0%

Secondary Endpoints

TIMI Grade 2 or 3 Flow at 90 Minutes.

The percentage of subjects with TIMI grade 2 or 3 flow appears to increase minimally with increasing doses of TNK (Table 8); overall, the rates for TNK are consistent with that of t-PA.

Table 8: TIMI Grade 2 or 3 Flow at 90 minutes: efficacy evaluable cohort

	TNK			t-PA
	30 mg	40 mg	50 mg	100 mg
N	302	148	76	311
Number of subjects (%)	232 (76.8%)	117 (79.1%)	67 (88.2%)	254 (81.7%)
95% CI	(71.6%, 81.5%)	(71.6%, 85.3%)	(78.7%, 94.4%)	(76.9%, 85.8%)

CBER Exploratory Analysis on TIMI Grade Flow

To provide insight into changes in IRA flow with respect to time, CBER organized TIMI grade flow data into shift tables. The analysis is limited to the 432 subjects for whom arteriographic data were successfully obtained at all three time points (60, 75 and 90 minutes). This encompasses roughly half of the efficacy evaluable population.

Shifts in TIMI grade flow between 60 and 90 minutes are summarized by treatment group in Figure 1. Sixty- and 90-minute data are depicted in rows and columns, respectively. (TIMI flows at 75 minutes are not included in this analysis.) Within each treatment group array, cells towards the lower left represent reductions in TIMI flow during the 60 to 90 minute interval post-treatment, such as might result from an insufficient lytic state, or from decreasing thrombolysis as the thrombolytic agent is cleared. Cells towards the upper right represent improvements in TIMI flow with time, implying maintenance of a milieu favoring continued thrombolysis.

Two (2) subjects in the t-PA group and no subjects in the 50 mg TNK group exhibit decreases in TIMI flow between 60 and 90 minutes. Results for the 40 mg TNK group appear similar, with 4 subjects showing reductions in TIMI flow with time. Casual inspection of the shift table from the 30 mg TNK group suggests that there are greater numbers of subjects with decreases in TIMI flow in this group. However, the tables display raw numbers, and there are approximately twice as many subjects in the 30 mg TNK group as in the 40 mg TNK group. Comparison of the 30 mg TNK group to the t-PA group is more informative, because there are similar numbers of subjects in each. In this comparison, there do appear to be excess subjects in the 30 mg TNK group who exhibit decreases in TIMI flow with respect to time. This suggests that the 30 mg TNK dose, as administered in this study population, was less effective than t-PA in *maintaining* thrombolysis. Both the 40 mg TNK dose, and particularly the 50 mg TNK dose, appear similar to t-PA in this regard.

Figure 1: CBER Exploratory Analysis: Changes in TIMI Grade Flow – 60 to 90 Minutes

TNK 30 mg

60-Minute TIMI Flow \ 90-Minute TIMI Flow	0	1	2	3
0	26	1	2	3
1	0	2	0	1
2	4	4	28	12
3	2	1	7	69

TNK 40 mg

60-Minute TIMI Flow \ 90-Minute TIMI Flow	0	1	2	3
0	9	1	1	2
1	1	4	1	1
2	0	1	10	7
3	1	0	1	40

TNK 50 mg

60-Minute TIMI Flow \ 90-Minute TIMI Flow	0	1	2	3
0	5	1	0	3
1	0	0	0	0
2	0	0	9	4
3	0	0	0	19

t-PA 100 mg

60-Minute TIMI Flow \ 90-Minute TIMI Flow	0	1	2	3
0	19	2	3	2
1	0	3	0	0
2	2	0	27	22
3	0	0	0	69

Numbers in the arrays refer to numbers of subjects.

Corrected TIMI Frame Count (CTFC) – 90 Minutes

Table 9 summarizes 90-minute CTFC by treatment group. Lower CTFC indicates more rapid flow. For calculation of median scores, an imputed score of 100 was assigned for occluded vessels. Median frame counts are presented, as well as dichotomized variables for CTFC < 28 (yes/no) and CTFC < 40 (yes/no).

Table 9: Corrected TIMI Frame Count (CTFC) at 90 Minutes

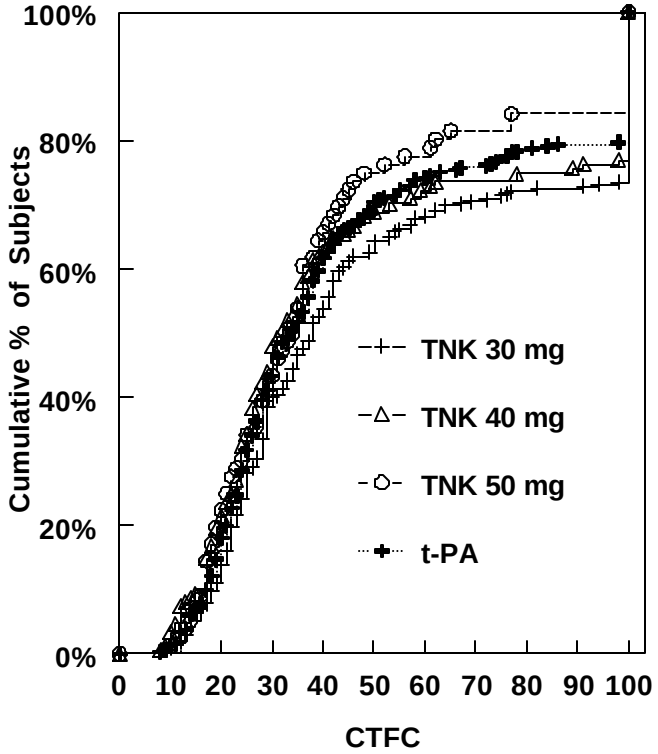
	TNK 30 mg	TNK 40 mg	TNK 50 mg	t-PA
N	297	147	74	306
Median TIMI Frame Count	38	32	34	34
subjects with TFC < 28 (N, %)	92 (31%)	60 (41%)	27 (36%)	113 (37%)
subjects with TFC < 40 (N, %)	159 (54%)	92 (63%)	49 (66%)	186 (61%)

The 40 mg TNK, 50 mg TNK and t-PA groups appear similar in median CTFC; however, median CTFT tended to be higher in the 30-mg TNK group compared to the other groups. The proportions of subjects achieving CTFCs of < 28 and < 40 tended to be somewhat lower in the 30 mg TNK group, accordingly.

Figure 2 presents the cumulative distribution of 90-minute CTFCs for all treatment groups. Shifts in the curves towards the left indicate a greater proportion of subjects with lower CTFC, i.e., a better result.

The distributions suggest improving efficacy with increasing doses of TNK. The 40 and 50 mg TNK groups appear to bracket the t-PA group. The 30 mg TNK group has the least favorable distribution.

Figure 2: 90-Minute CTFC – Cumulative Distribution



Exploratory Subgroup Analyses on TIMI Frame Counts at 90 minutes

Using 90-minute angiographic data, CBER assessed the proportions of subjects with TIMI frame counts < 28 for subgroups of age, gender, weight, "symptom-to-needle" time, IRA and time of study entry (relative to the 9/19/96 protocol amendment). The numbers of subjects in each treatment arm/subgroup are limited, but results appear generally consistent across subgroups (data not shown). Again, a trend towards less favorable TIMI frame counts is evident in the 30 mg TNK group.

TIMI Flow at 60 and 75 Minutes

The sponsor summarized the number and percentage of subjects with TIMI grade 3 flow and TIMI grade 2 or 3 flow at 60 and 75 minutes (data not shown), and found the results to be similar across treatment groups.

Reviewer's Comment(s): Performance of coronary 60- and 75-minute arteriography was not protocol-mandated, but was to be performed "when feasible." Thus, substantial numbers of subjects did not undergo arteriography and did not contribute data at all time points, such that data were not directly comparable across time.

CBER Exploratory Analysis of Corrected TIMI Frame Count by Time

CBER performed an analysis of CTFC by time, including only subjects with a complete set of CTFC data (60, 75 and 90 minutes, Table 10). This included 52% of all subjects in the efficacy evaluable population. Serial assessment of CTFC provides insight into the rapidity of restoration of IRA flow. For the 30 mg TNK group, median CTFC and the proportions of subjects with CTFC < 28 and < 40 remain relatively constant through 60, 75 and 90 minutes. In contrast, CFTCs tend to improve between 60 and 90 minutes in the 40 and 50 mg TNK groups. For t-PA, there is no apparent change in CTFC between 60 and 75 minutes, but improvement is apparent between 75 and 90 minutes. These data suggest that subjects

treated with 40 and 50 mg TNK achieve angiographic reperfusion no less rapidly than subjects treated with t-PA.

Table 10: Corrected TIMI Frame Count (CTFC) by Time – 60, 75, and 90 Minute Data

	TNK 30 mg	TNK 40 mg	TNK 50 mg	t-PA
N	163	79	41	149
60 minutes				
Median CTFC	39	37	39	41
Subjects with CTFC < 28 (N, %)	47 (29%)	24 (30%)	13 (32%)	41 (28%)
Subjects with CTFC < 40 (N, %)	82 (50%)	41 (52%)	21 (51%)	71 (48%)
75 minutes				
Median CTFC	40	34	33	41
Subjects with CTFC < 28 (N, %)	48 (29%)	27 (34%)	14 (34%)	37 (25%)
Subjects with CTFC < 40 (N, %)	81 (50%)	44 (56%)	23 (56%)	72 (48%)
90 minutes				
Median CTFC	38	32	32	34
Subjects with CTFC < 28 (N, %)	52 (32%)	31 (39%)	15 (37%)	58 (39%)
Subjects with CTFC < 40 (N, %)	86 (53%)	50 (63%)	26 (63%)	89 (60%)

Reocclusion at 18–36 Hours

Reocclusion rates were assessed at 18–36 hours in subjects at selected study sites who had a TIMI grade 2 or 3 flow reading at 90 minutes and who did not have PTCA. Reocclusion was defined as a TIMI grade flow of 0 or 1 at the follow-up visit as determined by the study site. As shown in Table 11, 36-hour data were collected for ~17% of subjects. Reocclusion was infrequent in all treatment groups.

Table 11: Reocclusion at 18-36 hours

	TNK 30 mg	TNK 40 mg	TNK 50 mg	t-PA
N	232	117	67	254
No. of subjects with 18- to 36-hour arteriogram	58	22	10	60
No. of subjects (%) with TIMI 0 or 1 at 18-36 hours (investigator reading)	2 (3.4%)	1 (4.5%)	1 (10.0%)	2 (3.3%)

Pharmacokinetics

Of the 103 PK/PD subjects enrolled to receive one of three doses of TNK in this trial, PK data were available for 99 subjects (the sponsor excluded data from 4 subjects in the 30 mg group with aberrant plasma concentrations). Data regarding weight, height, age and gender were available for 96 of 99 subjects, and statistical analyses to examine the relationship between subject pharmacokinetics and demographics were conducted for these subjects.

Mean maximum plasma TNK concentration (C_{max}) increased in a dose-proportional manner (7.5–11.6 µg/mL) following IV bolus administration in the 30 to 50 mg dose range. Following a

Table 12: PK Parameters by Dose Regimen

	TNK 30 mg (n=48) [a]	TNK 40 mg (n=31)	TNK 50 mg (n=20)	t-PA (n=53) [b]
CL (mL/min)	98.5 ± 42	119 ± 49	99.9 ± 32	453 ± 170
C_{max} (μg/mL)	7.5 ± 5.2	9.5 ± 8.1	11.6 ± 4.4	4.3 ± 3.4
C(0) (μg/mL)	10.0 ± 7.3	10.9 ± 11	15.2 ± 12	—
V_1 (L)	4.2 ± 2.6	5.4 ± 2.7	4.7 ± 2.6	7.2 ± 4.1
V_{ss} (L)	6.3 ± 3.3	8.0 ± 5.9	6.1 ± 2.4	28.9 ± 22
MRT (min)	62.1 ± 15	63.1 ± 22	60.3 ± 12	61.0 ± 39 [c]
$t_{1/2}$ Initial (min) [d]	21.5 ± 8.2	23.8 ± 5.5	20.1 ± 10	—
$t_{1/2}$ Terminal (min) [d]	116 ± 63	129 ± 87	90.4 ± 35	144 ± 100

[a] Data from 4 subjects were omitted because of aberrant plasma concentrations, with the exception of C_{max} for 30-mg TNK, where n=52.

[b] Pharmacokinetics for t-PA were calculated using a non-compartmental analysis. Data from 3 subjects were not fit because of aberrant plasma concentrations.

[c] A corrected mean residence time (MRT) for the t-PA group was calculated based on the rate and duration of infusion.

[d] Half-lives were calculated using only data from subjects exhibiting biexponential PK.

bolus dose of TNK, the plasma concentrations in most subjects decreased in a biphasic manner. The initial and terminal phase half-lives were approximately 22 minutes and 90 – 129 minutes, respectively. The initial volume of distribution (V_1) ranged from 4.2 to 5.4 liters, approximating plasma volume, but was highly variable. The steady-state volume of distribution (V_{ss}) was slightly larger than V_1, suggestive of extravascular distribution or tissue binding. The mean residence time (MRT) in the body was approximately 1 hour. TNK plasma clearance (CL) was approximately 100 mL/min and was similar for the 30, 40 and 50 mg doses. The estimated initial half-life of TNK (20 – 24 minutes) was approximately 5-fold longer than that reported for t-PA (3–5 minutes) and clearance was approximately 4-fold slower than that observed for t-PA (~105 versus ~450 mL/min, respectively). For TNK-treated subjects, subject weight accounted for 19% of the variability in clearance, suggesting that weight was an important covariate. For example, there was a 9.6 mL/min increase in CL for a 10-kg increase in subject weight. Subject age accounted for an additional 11% of the variability in TNK plasma clearance. On average, TNK clearance was lower (by 19.2 mL/min) in women compared with men, although this difference was not statistically significant and may have been related to the mean 12 kg weight difference between men and women. Because TNK was analyzed using compartmental pharmacokinetics and t-PA was analyzed using non-compartmental pharmacokinetics, a full PK comparison between TNK and t-PA was not performed.

Measures of coagulation and fibrinogenolysis

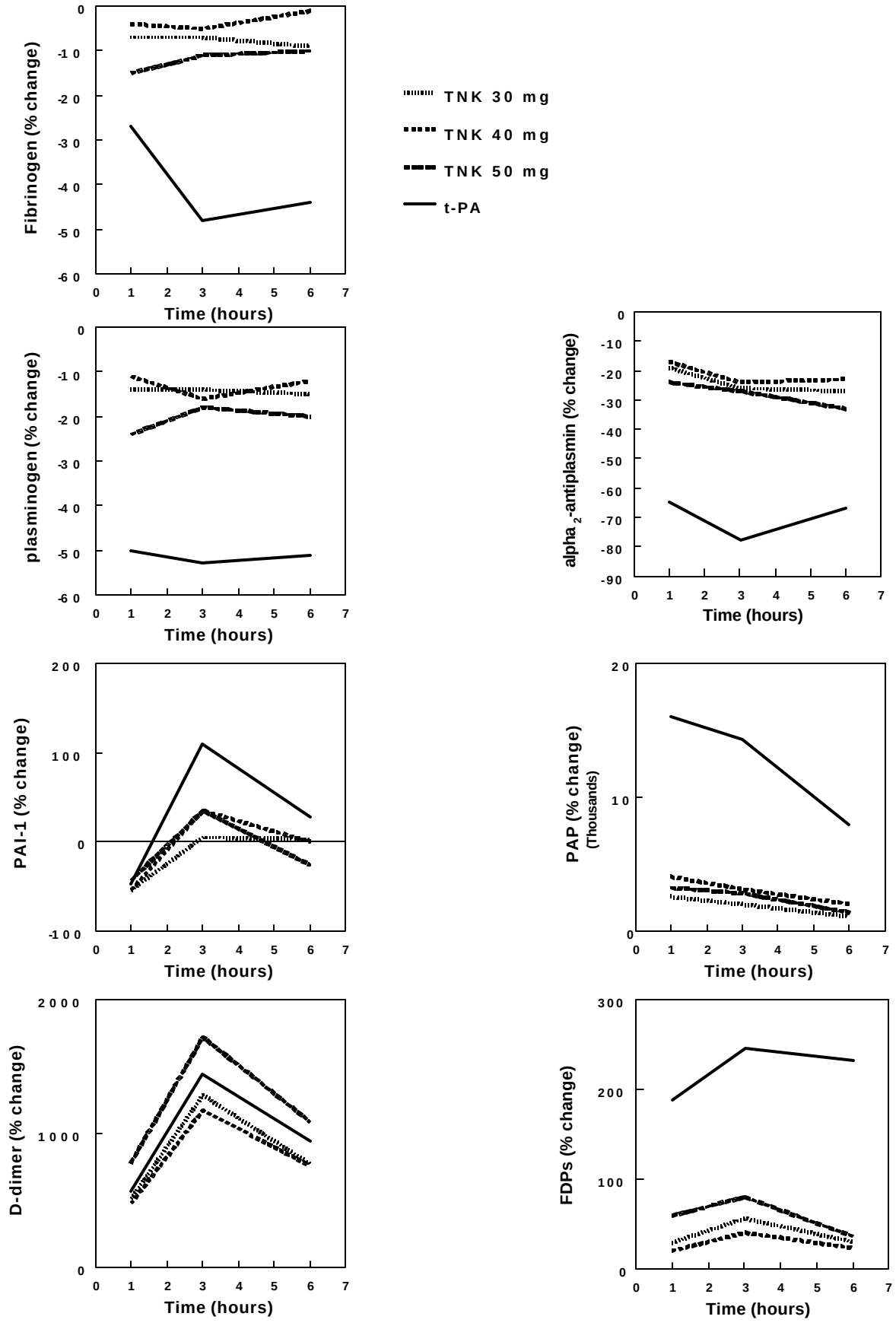
There were notable differences between TNK and t-PA with respect to levels of fibrinogen, plasminogen, α_2-antiplasmin (the fluid-phase inhibitor of plasmin), plasmin-α_2-antiplasmin complex (PAP), and fibrin degradation products (FDPs) (Figure 3, Table 13). The median decrease in fibrinogen levels was approximately 15% over the first 6 hours in TNK-treated subjects, compared to 44% in subjects who received t-PA. Similarly, median decreases in plasminogen levels were < 25% for subjects who received TNK, compared to > 50% in subjects treated with t-PA. Alpha-2-antiplasmin, a sensitive gauge of plasmin generation, decreased by only 20 - 30% in TNK-treated subjects, compared to 65 - 67% in t-PA-treated subjects. Fibrin degradation products were increased substantially in subjects treated with t-PA, with a median increase of 246% at 1 hour, in comparison to a median increase ranging from 20%–59% in subjects treated with TNK.

Table 13: Median % Change in Coagulation Parameters

	TNK-tPA 30 mg (n=52)	TNK-tPA 40 mg (n=31)	TNK-tPA 50 mg (n=20)	t-PA (n=56)
1 Hour				
Fibrinogen	-7%	-4%	-15%	-27%
Plasminogen	-14%	-11%	-24%	-50%
a2-Antiplasmin	-19%	-17%	-24%	-65%
PAI-1	-54%	-54%	-43%	-47%
PAP	2563%	4061%	3245%	15978%
D-dimer	513%	477%	782%	571%
FDPs	29%	20%	59%	188%
3 Hours				
Fibrinogen	-7%	-5%	-11%	-48%
Plasminogen	-14%	-16%	-18%	-53%
a2-Antiplasmin	-26%	-24%	-27%	-78%
PAI-1	5%	35%	35%	110%
PAP	1993%	3126%	2832%	14322%
D-dimer	1287%	1173%	1721%	1449%
FDPs	56%	40%	80%	246%
6 Hours				
Fibrinogen	-9%	-1%	-10%	-44%
Plasminogen	-15%	-12%	-20%	-51%
a2-Antiplasmin	-27%	-23%	-33%	-67%
PAI-1	3%	0%	-26%	28%
PAP	1091%	2009%	1381%	7923%
D-dimer	771%	759%	1088%	947%
FDPs	30%	23%	36%	232%

Median plasminogen activator inhibitor-1 (PAI-1) levels were decreased ~50% from baseline at 1 hour, rebounded above baseline at 3 hours, and returned to near-baseline at 6 hours for subjects who received both TNK and t-PA. Median levels of PAP complexes increased 3 – 5 times as much 1 hour after treatment with t-PA compared with TNK. D-dimer levels increased substantially within 1 hour after treatment; levels were comparable in the TNK and t-PA treatment groups.

Figure 3: Effect of TNK and t-PA on Coagulation and Fibrinogenolysis



Safety

Clinical Outcomes

There were 42 deaths within 30 days of study agent administration. Thirty-day mortality rates ranged from 3.6% to 6.5% for the 4 treatment groups, typical of the AMI patient population (Table 14). The composite endpoint of death or non-fatal stroke was not statistically different across the treatment groups, and ranged from 3.9% to 6.5%. Likewise, there were no apparent inter-group differences with respect to ICH, fatal ICH or total stroke. Rates of ICH tend to be higher than expected on the basis of previous studies, although no conclusions can be drawn because of limited sample size and low numbers of observed events.

Reviewer's Comment(s): This was an angiographic study that was not powered to assess differences in mortality rates or CNS events.

Rates of other principal clinical outcomes are also summarized by treatment group in Table 14. Differences were not significant between groups with respect to pulmonary edema, cardiogenic shock, revascularization, recurrent MI, or anaphylaxis.

Table 14: Clinical Outcomes at 30 Days

	TNK 30 mg	TNK 40 mg	TNK 50 mg	t-PA
N	308	154	78	316
Death	11 (3.6%)	10 (6.5%)	3 (2.6%)	18 (5.7%)
Composite of Death or Non-Fatal Stroke	12 (3.9%)	10 (6.5%)	4 (5.1%)	18 (5.7%)
Total Stroke	6 (1.9%)	4 (2.6%)	4 (5.1%)	9 (2.8%)
ICH	3 (1.0%)	3 (1.9%)	3 (3.8%)	6 (1.9%)
Fatal ICH	1 (0.3%)	3 (1.9%)	2 (2.6%)	2 (0.6%)
Pulmonary Edema (Killip Class III)	17 (5.5%)	7 (4.5%)	1 (1.3%)	13 (4.1%)
Cardiogenic Shock	8 (2.6%)	5 (3.2%)	1 (1.3%)	13 (4.1%)
Revascularization	215 (71.2%)	101 (68.2%)	45 (59.2%)	200 (64.3%)
Recurrent MI	16 (5.2%)	10 (6.5%)	2 (2.6%)	18 (5.7%)
Anaphylaxis	0	0	0	1 (0.3%)

Bleeding Events

Bleeding event rates must be considered in light of the open-label study design, with the recognition that subjective factors enter into decisions to categorization of events as “serious.” Moreover, the ramifications of the protocol amendment must be considered when interpreting these data. The effect of the amendment was to discontinue the 50 mg TNK group, initiate a 40 mg group, and institute measures to decrease the risk of bleeding (guidelines for heparin administration were altered, abciximab use was proscribed and the upper age limit for study inclusion was deceased).

The rates of serious bleeding appear to increase with increasing doses of TNK (Table 15), although, for reasons noted above, the rates are not directly comparable across groups. The rate of all serious bleeding events in the t-PA group (8.5%) was bracketed by the rates in the 40 and 50 mg TNK groups. Fewer subjects required transfusion in the 30 and 40 mg TNK groups, compared with the t-PA group. Catheter site-related bleeding accounted for roughly 25% of all serious bleeding events in TNK-treated subjects, and 37% of all serious bleeding in the t-PA group.

Table 15: Serious Bleeding Events

	TNK 30 mg	TNK 40 mg	TNK 50 mg	t-PA
N	308	154	78	316
At least 1 event	6 (1.9%)	8 (5.2%)	9 (11.5%)	27 (8.5%)
Hemorrhage				
retroperitoneal	0	0	0	3 (0.9%)
catheter site	1 (0.3%)	2 (1.3%)	3 (3.8%)	10 (3.2%)
ICH	3 (1.0%)	3 (1.9%)	3 (3.8%)	6 (1.9%)
"cardiovascular"	1 (0.3%)	0	0	2 (0.6%)
GI	0	0	1 (1.3%)	4 (1.3%)
anemia	0	2 (1.3%)	0	0
required transfusion	3 (1.0%)	2 (1.3%)	3 (3.8%)	22 (7.0%)

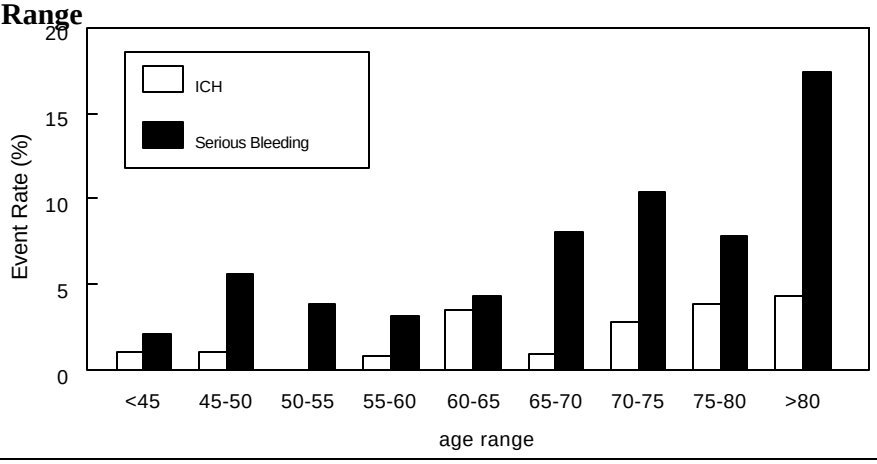
The sponsor asserts that "the incidence of bleeding events appeared to decrease following the protocol amendment, suggesting that factors changed by the protocol amendment, including heparin administration, may play a role in serious bleeding incidence." This statement is applicable only to the 30 mg TNK group and the t-PA group, because the 50 and 40 mg TNK groups enrolled subjects exclusively before and after amendment, respectively. This statement has, at its basis, the following observations: 1) In the 30 mg TNK group, there were 3 ICH events and 3 other serious bleeding events in the 134 subjects treated before protocol amendment, compared with no ICH or other serious bleeding events in 174 subjects treated after protocol amendment; and 2) there were 4 ICH and 11 other serious bleeding events in 143 subjects in the t-PA group treated prior to protocol amendment, with 2 ICH events and 10 other serious bleeding events in 173 subjects treated after the amendment.

Reviewer's Comment(s):
Although rates of serious bleeding events did appear to decrease after protocol amendment, it is not known to what extent, if any, if the protocol changes are responsible for this trend. Moreover, the specific effect of the change in heparin administration guidelines, if any, is unknown.

CBER attempted to identify specific factors, modified as a result of protocol amendment, that may have been importantly associated with bleeding events. Thus, CBER reviewed the 6 subjects in the 30 mg dose group with serious bleeding events prior to protocol amendment, and considered how the protocol amendment would have affected their enrollment and/or management:

- Age: There appears to be a relation between age and serious non-ICH bleeding events, and possibly an association between age and ICH events (Figure 4). However, none of the subjects with serious bleeding events was ≥ 80 years-old. Thus, age was not a factor in the apparent

Figure 4: CBER Analysis of ICH and Serious Bleeding by Age Range



decrease in serious rates of bleeding before versus after protocol amendment.

- Abciximab: Abciximab use, although proscribed by protocol amendment, was fairly evenly divided before and after amendment. None of the subjects with a serious bleeding event actually received abciximab.
- Heparin: One of the 6 subjects in the 30-mg dose group with a serious bleeding event prior to protocol amendment (a non-ICH bleeding event) weighed < 67 kg, and would have received a reduced heparin dose if enrolled after protocol amendment. Four subjects received additional heparin boluses, subsequently restricted by protocol amendment. Thus, for these 6 subjects, the administration of additional heparin boluses appears to be an identifiable risk factor that would have been affected by the changes in the protocol. This association does not prove, however, that curtailment in heparin administration affected serious bleeding rates.

CBER performed a multivariate analysis of serious bleeding events using the terms gender, age (as a continuous variable), abciximab use (at any time, yes/no), heparin bolus >2500 units on ≥ 2 occasions during hospitalization (yes/no) and heparin bolus >2500 units on ≥ 2 occasions on the same or consecutive days (yes/no). Whereas gender, age and abciximab use were significantly associated with severe bleeding events, there was no significant association between severe bleeding events and heparin use (by total bolus doses received, total bolus doses > 2500 units within the same day, or total bolus dose per kg). Thus, there is little support for the assertion that changes in heparin administration guidelines, per se, were responsible for decreasing the incidence of serious bleeding events.

Serious Non-Bleeding Adverse Events

Approximately 31% of subjects experienced at least one serious, non-bleeding adverse event (Table 16). Events in the cardiovascular system (25%) and in the body as a whole (5%) occurred most frequently. The percentage of subjects experiencing at least one event was higher among the 30 mg TNK (34%) and 40 mg TNK dose groups (38%) compared with t-PA (26%).

Coronary occlusions and angina pectoris were generally more frequent in TNK subjects than t-PA subjects. Subjects treated with 40-mg TNK also experienced more angina pectoris than did tPA subjects.

Table 16: Serious Non-Bleeding Adverse Events Reported in >2% of the Study Population

	TNK 30 mg	TNK 40 mg	TNK 50 mg	t-PA
N	308	154	78	316
Subjects with ≥ 1 event N (%)	105 (34.1%)	58 (37.7%)	18 (23.1%)	81 (25.6%)
Chest pain	8 (2.6%)	4 (2.6%)	4 (5.1%)	3 (0.9%)
Coronary occlusion	27 (8.8%)	7 (4.5%)	2 (2.6%)	7 (2.2%)
Heart arrest	6 (1.9%)	7 (4.5%)	0	8 (2.5%)
Myocardial infarct	16 (5.2%)	7 (4.5%)	1 (1.3%)	11 (3.5%)
Ventricular fibrillation	7 (2.3%)	7 (4.5%)	3 (3.8%)	9 (2.8%)
Shock	6 (1.9%)	4 (2.6%)	1 (1.3%)	11 (3.5%)
Angina pectoris	15 (4.9%)	8 (5.2%)	0	3 (0.9%)

Non-Serious Adverse Events

Bleeding Adverse Events

Roughly three-quarters of the subjects in each group reported non-serious bleeding adverse events, primarily catheter-site hemorrhage, hematuria and ecchymoses. There were no apparent differences between treatment groups.

Non-Bleeding Adverse Events

For all groups, non-serious non-bleeding-related AEs were reported in ~ 90% of subjects. AEs were generally reported with similar frequencies in all groups, although there were a few exceptions: There tended to be excess nervous system AEs in the 50-mg TNK group, principally due to excesses in reported confusion, insomnia, anxiety and dizziness. There appeared to be excess respiratory AEs reported in the 40-mg TNK group. These included increased cough, dyspnea, pleural effusion and "lung disorder." Given the overall frequency of these events in a hospitalized AMI population and the multiplicity of analyses, these differences, particularly if they are not recapitulated in ASSENT I and ASSENT II, are most likely due to play of chance.

Laboratory Values

Values for BUN, creatinine, glucose, hemoglobin, hematocrit, white blood cell count, platelet count and electrolytes were typical of a hospitalized acute MI patient population and similar in all groups.

Incidence of Anaphylaxis; Antibody Formation Against TNK

There were no anaphylactic events. There was one non-serious anaphylactoid event in the 30 mg group. Anti-TNK antibody results were available for 73% of TNK-treated subjects (395/543) at 30 days. One subject had a positive titer at 30 days, that was subsequently negative at 90 days. A number of subjects had positive titers at baseline that reverted to negative at 30 days.

Relation Between Weight-Adjusted Dose and Activity

The sponsor conducted exploratory analyses to evaluate the relation between weight-adjusted dose, TIMI grade flow and TIMI frame count. Ultimately, the results of these analyses formed part of the basis for the TNK dosing regimen for the phase 3 study (ASSENT II). For TNK-treated subjects, the relation between TIMI grade flow, dose and weight was evaluated using a logistic regression model, with TIMI grade flow as the dependent variable. Log [dose/weight] was found to be associated with TIMI grade flow, and was a significant predictor of TIMI grade flow even when covariates such as infarct location and age were included in the model.

The sponsor also constructed cumulative distributions of 90-minute CTFCs by dose/weight quartiles, recapitulated by CBER (Figure 5). There appears to be a trend of more favorable CTFC with increasing dose/weight. Of note, the mg/kg proportionality used to establish the dosing paradigm for ASSENT II was 0.53, which lies between the 50^{th} and 75^{th} percentile in this study (weight-adjusted dose of 0.444-0.545 mg/kg, open circles).

The sponsor also performed these analyses for the 30, 40 and 50 mg TNK dosing groups, separately. Whereas the distributions for the 40 and 50 mg groups show only marginal trends in improved patency with increasing weight-adjusted dose quartile, there is a conspicuous separation of the distributions for the 30 mg dosing cohort (Figure 6). The principal conclusion is that a TNK dose of 30 mg is insufficient for higher-weight subjects, whereas the 40 and 50 mg TNK doses are more likely to be adequate for subjects of all weights.

TIMI 10B Summary

This was a 880-subject angiographic study comparing 30, 40 and 50 mg TNK and accelerated t-PA. Coronary cineangiography (TIMI flow and CTFC) provided direct assessment of IRA patency, with a primary endpoint of 90-minute TIMI Grade 3 Flow.

Ninety-minute TIMI flow data were obtained in 98% of subjects. Serial angiographic data (60, 75 and 90 minutes after initiation of treatment) were available in 52% of subjects. Delayed angiographic data (18 – 36 hours) were available to assess reocclusion in 17% of subjects.

The data suggest that single 40 and 50 mg bolus doses of TNK are comparable to accelerated t-PA in achieving arteriographic reperfusion at 90 minutes. The effects of TNK appear to be dose-related; the 30 mg TNK dose appears to be less effective than 40 or 50 mg TNK or accelerated t-PA in achieving arteriographic reperfusion.

Data from the subset of subjects for whom serial angiographic results were available at the all time points (60, 75 and 90 minutes) suggest that subjects treated with 40 and 50 mg TNK achieve

Figure 5: 90-Minute CTFC in All TNK-Treated Subjects – Cumulative Distributions for Weight-Adjusted Dose Quartiles

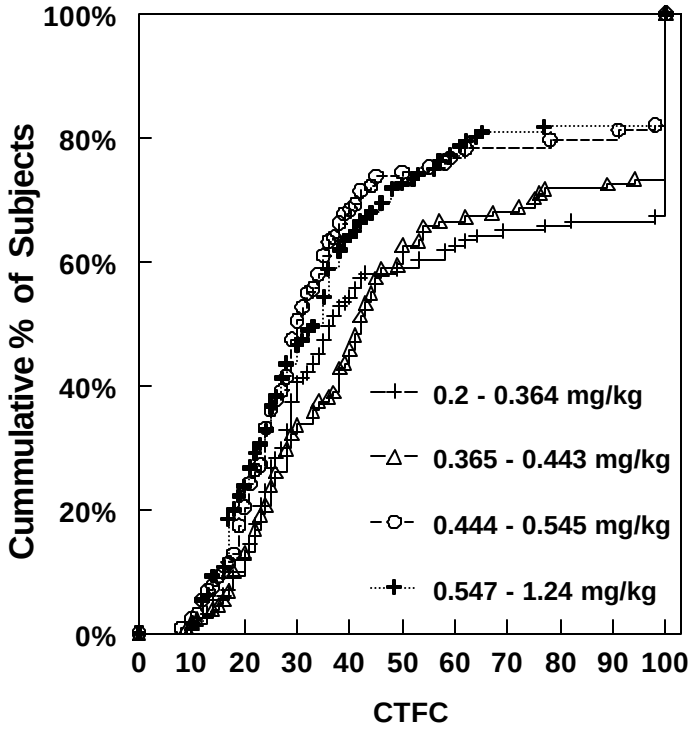
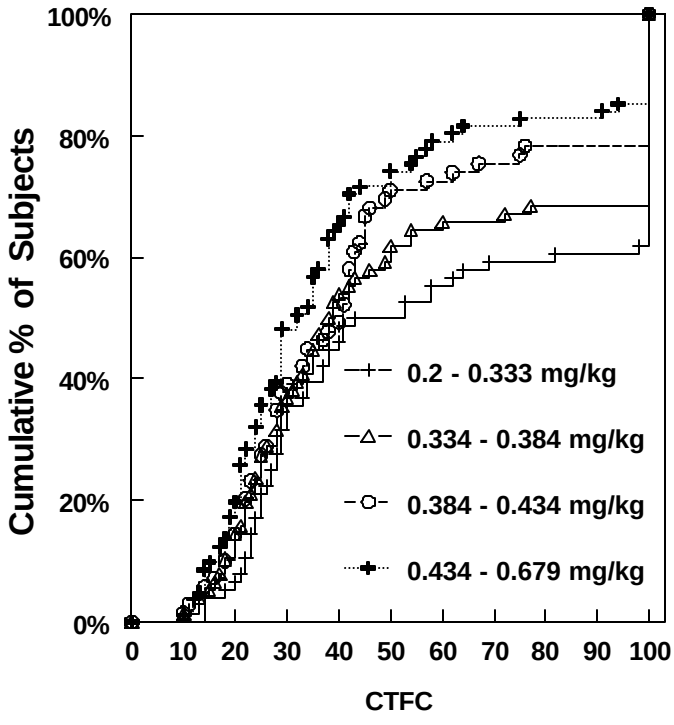


Figure 6: 90-Minute CTFC in 30 mg TNK-Treated Subjects – Cumulative Distributions for Weight-Adjusted Dose Quartiles



angiographic reperfusion no less rapidly than subjects treated with t-PA. Reocclusion at 18 – 36 hours was rare.

Exploratory analyses of the relationship between weight, weight-adjusted dose, TIMI grade flow, and TIMI frame count suggest that that weight-adjusted dose is predictive of patency, and provide a rationale for weight-adjusted dosing in future studies.

The estimated initial half-life of TNK was 20 – 24 minutes, approximately 5-fold longer than that of t-PA (3 – 5 minutes). TNK caused considerably less depletion of circulating fibrinogen and plasminogen than t-PA, although the clinical significance of these observations is unknown.

No anaphylaxis was reported. Anti-TNK antibody results were available for 395 TNK-treated subjects at 30 days. One subject had a positive titer at 30 days, that was subsequently negative at 90 days.

Deaths and ICH events were too infrequent to draw conclusions regarding disparities between TNK and t-PA, or between disparate TNK dosing cohorts. Overall, mortality rates were comparable to recent large trials of thrombolytics in AMI. Rates of ICH appeared slightly high for this patient population (15 in 856 subjects, 1.8%), but no definitive conclusion can be drawn given the size of this study. The safety profile of TNK appears similar to that of other thrombolytic agents, with bleeding the major safety concern. Overall, the data suggest that TNK, administered as an IV bolus at doses of 40 and 50 mg, is similarly effective to accelerated t-PA with respect to coronary thrombolysis and safety.

Protocol N0683g – ASSENT I

Title:	A Phase II, Randomized, Open-Label, Parallel-Group, Multicenter, International Trial of the Safety of TNK-TPA in Acute Myocardial Infarction: ASSENT I (Assessment of the Safety of a New Thrombolytic: TNK-tPA)
Study Period:	March 1996 to April 1997
Funding:	Boehringer Ingelheim GbmH, Germany; Genentech, Inc., USA
Size:	3301 subjects at 288 sites in 20 countries

Dose Selection

ASSENT I was conducted in parallel with the angiographic TIMI 10B study, and the rationale for TNK dose selection was identical (see page 12).

Objectives

The stated primary study objective was to evaluate the safety of several doses of TNK administered as a single bolus in subjects with AMI presenting within 12 hours after symptom onset.

Study Design

Overview

This Phase 2 investigation was a safety study – a randomized, open-label, multicenter, international, parallel group trial of TNK in subjects with AMI presenting within 12 hours of symptom onset. The study was planned to randomize 3000 subjects 1:1 to TNK 30 or 50 mg as an IV bolus. However, because of concerns regarding 3 ICH events in the 50 mg TNK dose

group early in the course of TIMI 10B, the 50 mg dose was replaced with a 40 mg dose in November 1996. This change was made when enrollment had reached ~10% of completion. The primary safety variable was ICH. Other principal variables included death, stroke, and bleeding events.

Administrative Structure/Data and Safety Monitoring Board

Genentech had primary responsibility for the North American sites; Boehringer Ingelheim had primary responsibility for non-North American sites. The trial was performed concurrently with TIMI 10B. A single Data and Safety Monitoring Board (DSMB), comprised of 4 independent cardiologists and an independent biostatistician, monitored both ASSENT I and TIMI 10B, as they enrolled concurrently.

Patient Population

The patient population included subjects with AMI who presented within 12 hours after symptom onset.

Inclusion Criteria

- ischemic discomfort ≥ 30 minutes in duration
- ST segment elevation ≥ 0.1 mV in two contiguous ECG leads indicative of AMI, or new LBBB with ST elevations ≥1 mm
- ability to be randomized within 12 hours after symptom onset
- age ≥ 18

Exclusion Criteria

- MI treated with any thrombolytic agent within the preceding 4 days
- arterial HTN defined as systolic BP > 180 mmHg and/or diastolic BP > 110 mmHg
- significant bleeding disorder within the past 6 months
- use of abciximab within 96 hours (added as result of 9/96 amendment)
- major surgery, biopsy of a parenchymal organ, or significant trauma within 3 months
- history of stroke or transient ischemic attack (TIA) or central nervous system (CNS) structural damage (i.e., neoplasm, aneurysm, intracranial surgery)
- oral anticoagulation - international normalized ratio (INR) ≥ 1.4; prothrombin time (PT) ≥ 14 seconds
- prolonged cardiopulmonary resuscitation (> 2 minutes) within 2 weeks
- noncompressible vascular puncture within 10 days
- pregnancy (positive urine pregnancy test) or lactation, parturition within the previous 30 days, or woman with childbearing potential not using adequate contraception
- other serious illness (e.g., active cancer, active infection)
- current cocaine abuse
- previous treatment with TNK

Treatment

Material Source

TNK and t-PA were provided as sterile, lyophilized powders for reconstitution with Sterile Water for Injection, USP (or EP for non–North American sites). Each TNK vial contained 20 mg of TNK and the following excipients: arginine, phosphoric acid, and polysorbate 20. The TNK vials were

to be reconstituted in 4 mL of Sterile Water for Injection (SWFI), resulting in a TNK concentration of 5 mg/mL.

Randomization

Randomization was blocked by study center. Treatment assignment was made through a centralized interactive voice response system.

Dose and Administration

All subjects were to receive TNK, administered as an IV bolus injection over 5 – 10 seconds. The originally planned doses of TNK were 30 and 50 mg. As noted above, the 50 mg dose was, in effect, replaced by a 40 mg dose after the September 1996 protocol changes.

Concomitant Medications

Specially Directed:

- Aspirin, 150-325 mg PO upon study entry, then 150 – 325 mg qd. Alternatively, 100 – 250 mg aspirin IV was acceptable where approved by local regulatory authorities. Subjects who had taken aspirin within 24 hours prior to randomization were to begin aspirin the following day.
- Heparin, 4000 units IV bolus, then 800 units/h infusion (subjects ≤ 67 kg); or 5000 units IV bolus, then 100 units/hour (subjects > 67 kg) for 48 – 72 hours. The bolus was to be administered as soon as possible after baseline aPTT was obtained. aPTT performed at baseline, 6, 12, 24 and 48 hours after the start of study agent, and daily thereafter while on IV heparin. Infusion adjusted to maintain aPTT at 55 – 80 seconds; aPTT assessed 6 hours after adjustments in infusion rate. After the September 1996 protocol changes, no additional heparin was to be given during diagnostic catheterizations on IV heparin. Additional, large heparin doses were to be avoided if rescue angioplasty was performed. If needed, additional heparin was administered in increments no larger than 2500 units.

Prohibited:

- abciximab during the first 96 hours after randomization (after September 1996 protocol changes)
- investigational medications or investigational devices through the 30-day follow-up period

Screening

Screening included medical history, physical examination (including Killip Class), 12-lead ECG, laboratory tests (CK/CK-MB, hematology, aPTT, chemistry panel, INR or prothrombin time, urinalysis, and pregnancy test (if applicable).

Monitoring

Electrocardiograms were repeated for signs and symptoms of recurrent myocardial infarction, and at discharge. Creatine phosphokinase (CPK) and MB isoenzymes were assessed at 8 and 12 hours and for signs suggestive of recurrent MI. Routine clinical laboratory studies were performed at 24 – 48 hours and at discharge. Activated PTT was assessed at 6 and 12 hours, 6 hours after adjustments in heparin dose, and daily while heparin was infused. Coagulation studies, including fibrinogen and fibrin degradation products (FDPs) or D-dimer, were assessed at 1 and 3 hours. A physical exam was performed at hospital discharge.

The following clinical outcomes were to be monitored and recorded: death, recurrent MI, stroke, (hemorrhagic, non-hemorrhagic, unknown), non-ICH bleeding complications, coronary

revascularization, pulmonary edema, cardiogenic shock and anaphylaxis. Subjects were to undergo immediate head CT or MRI for changes in neurologic function.

Reviewer's Comment(s): There was no assessment of anti-TNK antibodies in ASSENT I.

30-Day Follow-Up

Subjects were to be evaluated by telephone or visit between Days 30 and 37. Vital status, adverse events, clinical outcomes and re-hospitalization were to be reported for all subjects and captured in the CRFs.

Statistical Analysis Plan

The primary consideration of this study was an estimation of rates of ICH for various TNK doses. No true null hypothesis was tested, and much of the statistical plan was descriptive. Rates of stroke, death, recurrent MI, serious, life-threatening bleeding, pulmonary edema, cardiogenic shock, anaphylaxis and net clinical benefit were also estimated. Two-sided 95% confidence intervals were calculated for these event rates.

Interim Analysis

No formal, statistical interim analysis was planned. Safety was monitored on a continuous basis by a DSMB. By August 1996 there were 3 ICHs among the 78 subjects in the 50 mg TNK dose cohort in TIMI 10B. Although there were no ICHs among the 73 subjects in the 50 mg TNK group in ASSENT I, it was decided to suspend the 50 mg dose groups in both trials, and replace these groups with 40 mg cohorts.

Study Performance

Protocol Changes

The protocol was not formally amended, but was altered by three memoranda issued during the summer of 1996. On the basis of the initial July 1996 DSMB review of available data on 198 subjects and subsequent consideration of data by the Operations Committee, DSMB and Sponsor's Data Review Board, the following changes were implemented:

- The 50 mg TNK dose was discontinued.
- A 40 mg TNK dose was added.
- Concomitant medications that might influence the potential risk of major hemorrhage, including ICH, were restricted, including:
  - restriction of additional heparin use during catheterization and rescue angioplasty to increments of 2500 units, with a target activated clotting time (ACT) of 300 seconds
  - reduction of the initial heparin bolus for subjects weighing ≤ 67 kg; titration of heparin infusion to the aPTT was to begin at 6 hours
  - prohibition of the use of abciximab within 96 hours before and after randomization

ASSENT I Study Results

Patient Enrollment and Treatment Assignment

A total of 3301 subjects were enrolled at 20 sites. The US enrolled the largest number of subjects (588, 17.8%), followed by the Netherlands (340, 10.3%). Austria enrolled the smallest number of subjects (27, 0.8%), followed by the Czech Republic (33, 1.0%). The first subject

was enrolled on June 12, 1996, and enrollment was completed within a 10.5-month period. The enrollment rate increased throughout the first six study months, reaching a plateau of ~400 subjects per month until the last subject was enrolled on April 24, 1997. Among the 3301 subjects enrolled, 3235 subjects (98%) were actually treated: 1705 subjects received 30 mg TNK, 1457 received 40 mg TNK, and 73 received 50 mg TNK. These 3235 subjects comprise the as treated (safety evaluable) population, and are the focus of this report and analyses. The ITT population is less relevant with respect to safety, and is not discussed further.

The enrolled and safety evaluable populations are summarized in Table 17, with breakdowns by treatment group and timing of enrollment (with respect to the 9/96 protocol changes). Note that 90% of subjects were enrolled after the changes in the protocol were implemented:

Table 17: Enrolled and Safety Evaluable Subjects by Treatment Group

	TNK-tPA 30 mg	TNK-tPA 40 mg	TNK-tPA 50 mg	Total
Enrolled	1727	1498	76	3301
Treated (safety evaluable)	1705	1457	73	3235
Before Protocol Change	248	0	71	319
After Protocol Change	1457	1457	2	2916

Reviewer's Comment(s): Prior to the 9/96 protocol changes, subjects were assigned to 30 or 50 mg TNK in a 1:1 ratio; however, there is a marked imbalance between these groups (248 versus 73). This occurred because enrollment proceeded through September and October 1996 (the period when the changes were implemented), with virtually all subjects assigned to the 30 mg TNK group. Randomization to the 40 mg group did not begin in earnest until November 1996.

Protocol Deviations

At study entry, 28 subjects did not meet inclusion/exclusion criteria; however, of these, only 19 were in the safety evaluable population. One failed to provide informed consent, 11 had BP > specified limits, 6 had other contraindications to receiving thrombolytic therapy, and 1 was participating in another trial. Upon review at study completion, 36 subjects (~1%) were found to have conditions that did not meet inclusion criteria, and 86 (~2.6%) were found to have exclusion criteria.

Reviewer's Comment(s): In the context of a safety study, the enrollment of subjects who fail to meet inclusion criteria could decrease overall morbidity, thereby leading to an overestimation of safety. Conversely, subjects who violate exclusion criteria may have coexisting conditions that increase risk, and their enrollment could lead to underestimation of safety. For ASSENT I, the numbers of subjects who violated inclusion/exclusion criteria were probably too small to importantly affect the assessment of safety.

TNK was given as a single IV bolus over 5 – 10 seconds. Over 99% of subjects in the 30 and 40 mg groups received the treatment to which they were assigned; 93.2% of subjects in the 50 mg group received the assigned treatment.

Follow-Up

Of the 3235 subjects who received study drug, 2973 (91.9%) completed the 30-day follow-up, 208 (6.4%) died within 30 days of treatment, 1 withdrew because of an adverse event, 1 withdrew for non-medical reasons, 4 (0.1%) withdrew because of noncompliance, 13 (0.4%) withdrew for other reasons, and 35 (1.1%) were lost to follow-up.

Reviewer's Comment(s): For the study as a whole, there were 54 subjects (1.7%) who did not complete the 30-day follow-up. Because no specific statistical hypotheses were tested in this study, the loss of these subjects would not be expected to materially affect the results, and sensitivity analyses were not performed.

Study Population Baseline Characteristics

General

Baseline characteristics were typical of the AMI patient population and similar to the subject population in TIMI 10B: mean age was 61 years (range 23 to 94), with ~12% of subjects > 75 years-old. Roughly three-quarters of the subjects were male (Table 18). ASSENT I included a smaller proportion of subjects of African descent (1.7%) compared with TIMI 10B (4.9%). Anterior infarct was reported in 44.6% of subjects; 17% of subjects had a previous MI. Median "symptom-to-needle" time was 3.1 hours (range 23 minutes to 27.4 hours). Interestingly, 14 subjects were treated in the field, prior to hospital arrival. The 50 mg group tended to be slightly younger than the other groups; HR tended to be higher in the 40 mg group.

Reviewer's Comment(s): Presumably, the difference in proportions of subjects of African descent between ASSENT I and TIMI 10B relates to the disparity in enrollment from US sites (18% versus 56%, respectively). The differences in age and baseline HR are subtle, and not unexpected in a study of this size. They appear to be relatively unimportant, particularly when considered in light of the alterations in patient management mandated by the September 1996 protocol changes that differentially affected the 40 and 50 mg groups.

Concomitant and excluded medications

Concomitant medication use was similar across treatment groups. Overall, 96.4% of subjects received aspirin, and 98.9% of subjects received both a heparin bolus and infusion. Eighteen percent of subjects who received heparin received a bolus dose that was too high, based on the recommendations after the protocol changes. Twenty-one percent of subjects received an excessive heparin infusion dose.

Reviewer's Comment(s): Virtually all subjects in the 50 mg group were enrolled in advance of the protocol changes that curtailed heparin use. Thus, subjects in this group received excessive heparin compared to the other groups, with bolus and infusion doses exceeding recommendations in 40% and 33% of subjects, respectively.

On Days 0 and 1, IV nitrates and beta-blocker use was recorded commonly, in 67% and 62% of subjects, respectively, without significant differences between treatment groups. Angiotensin converting enzyme (ACE) inhibitor use was recorded in 25% of subjects; calcium antagonist use was reported in 14% of subjects. Use of ticlopidine and abciximab was uncommon (4.1 and 1.7% of subjects overall, respectively). Through hospital discharge, beta blockers were prescribed in 80.6% of all subjects, with metoprolol accounting for over half of all beta blockers.

Table 18: Demographic Characteristics and Baseline Disease Status (ASSENT I)

Characteristic	30 mg	40 mg	50 mg	Total
N	1705	1457	73	3235
mean age (SD) [years]	61.1 (12.5)	60.9 (12.4)	56.7 (12.1)	60.9 (12.4)
age > 75 [%]	12.8	11.1	6.8	11.9
number (%) male	1297 (76.1)	1104 (75.8)	56 (76.7)	2457 (76.0)
race [n (%)]				
Caucasian	1439 (84.4)	1195 (82.0)	58 (79.5)	2692 (83.2)
African descent	29 (1.7)	25 (1.7)	1 (1.4)	55 (1.7)
Asian	24 (1.4)	22 (1.5)	1 (1.4)	47 (1.5)
Hispanic	19 (1.1)	25 (1.7)	2 (2.7)	46 (1.4)
Native American	4 (0.2)	5 (0.3)	0	9 (0.3)
other and unspecified (French subjects)	190 (11.1)	166 (11.4)	11 (15.1)	386 (11.9)
mass [mean, SD, kg]	77.8 (14.4)	77.9 (14.0)	78.4 (15.1)	77.9 (14.3)
anterior infarct location [%]	759 (44.5%)	657 (45.1%)	26 (35.6%)	1442 (44.6%)
HR [mean, SD bpm]	75.4 (18.6)	76.8 (18.4)	74.8 (18.7)	76.0 (18.5)
systolic BP [mean, SD, mmHg]	135.5 (22.7)	135.3 (23.5)	133.1 (23.9)	135.4 (23.1)
previous medical history [%]				
HTN	34.3	37.1	35.6	35.6
previous MI	16.2	17.8	20.5	17.0
prior CABG	3.9	3.9	3.9	3.9
prior PTCA	5.1	5.6	5.1	5.6
Hx smoking	63.3	67.5	64.4	65.2
time to treatment [median, hours]	3.1	3.1	3.5	3.1

Efficacy

ASSENT I was not powered to assess mortality differences between treatment groups. For the study overall, the mortality rate was 6.4%, a rate typical of recent trials of thrombolytic agents in AMI. Mortality rates by treatment group are shown in Table 19. Historical mortality data for t-PA from GUSTO I are provided for reference (right column).

Reviewer's Comment(s): Mortality rates in the 30 and 40 mg groups are not directly comparable, due to protocol changes that affected the 90% of the 30 mg group and all of the 40 mg group. CBER calculated the mortality rate for subjects in the 30 mg group who enrolled after the protocol changes took effect (Table 19, second column from left). Thus, data from this subset of the 30 mg group are directly comparable with the 40 mg group. Paradoxically, mortality in the 30 mg group tended to *increase* after protocol changes were implemented to enhance safety. The reason for this is unclear; however, the numbers of events are limited, confidence intervals are wide, and the data can not rule out no difference.

Table 19: Mortality Rates by Treatment Group in ASSENT I, and Comparison with GUSTO I

	30 mg	30 mg (post 9/96)	40 mg	50 mg	Total ASSENT I	GUSTO I
N	1705	1475	1457	73	3235	10396
deaths (%)	117 (6.9%)	104 (7.1%)	88 (6.0%)	3 (4.1%)	208 (6.4%)	654 (6.3%)

Stroke

Thirty-day event rates for stroke, as classified by the Event Review Committee, are shown in Table 20. ICH events were reported in a total of 25 subjects, at an overall study rate of 0.9%. Although this rate is slightly higher than that reported for t-PA in GUSTO I (0.7%), ASSENT I included subjects who presented 6 – 12 hours after symptom onset, whereas GUSTO I enrolled subjects only through 6 hours after symptom onset. The rate of ICH for subjects in ASSENT I treated within 6 hours of symptom onset (0.6%) compares favorably with that of GUSTO I.

The ICH rates are 0.9% (95% CI: 0.5%, 1.5%) for the 30 mg group, 0.6% (0.3%, 1.2%) for the 40 mg group and 0 for the 73 subjects in the 50 mg group (0, 4.9%).

The sponsor notes that ICH rates were reduced after implementation of the September 24, 1996 protocol changes constraining heparin use and prohibiting abciximab within 96 hours of administration of study agent. Specifically, for the 30 mg group, ICH rates before and after the changes were 1.6% and 0.8%, respectively.

Table 20: 30-Day Stroke Rates – ASSENT I

	TNK-tPA 30 mg		TNK-tPA 40 mg		TNK-tPA 50 mg		Total	
N	1705		1457		73		3235	
	events	**%**	**events**	**%**	**events**	**%**	**events**	**%**
Total Strokes	26	1.5%	22	1.5%	0	0.0%	48	1.5%
Primary ICH	16	0.9%	9	0.6%	0	0.0%	25	0.8%
Ischemic Stroke	9	0.5%	14	1.0%	0	0.0%	23	0.7%
Hemorrhagic Conversion	1	0.1%	1	0.1%	0	0.0%	2	0.1%
Unclassified	1	0.1%	1	0.1%	0	0.0%	2	0.1%

some subjects had >1 stroke

Reviewer's Comment(s):

- Overall, there were 4 ICH events in 391 subjects enrolled before the protocol changes went into effect (event rate = 1.3%), and 21 ICH events in 2916 subjects enrolled after the changes (0.7%). Though the data do suggest a decrease in the ICH rate, no definitive conclusions can be reached regarding the effect of protocol changes on ICH, in light of the limited enrollment and small number of ICH events prior to the protocol changes.

- CBER assessed ICH rates in the contemporaneously randomized 30 and 40 mg groups (post protocol changes). For the 30 mg group, there were 14/1475 ICH events (0.9%), whereas there were 9/1457 events in the 40 mg group (0.6%).

Non-Stroke Major Clinical Outcomes

Non-stroke major clinical outcomes are listed in Table 21. Of note, the overall incidence of anaphylaxis is 0.1%

Reviewer's Comment(s): Though there appear to be some trends in the data (e.g., decreasing mortality rate and increasing incidences of pulmonary edema and cardiogenic shock with increasing doses of TNK), these differences must be considered in the context of the limited numbers of events overall, the very limited number of subjects in the 50 mg group and the multiplicity of analyses. Confidence intervals about the point estimates (not shown) are quite wide. Thus, not only are differences between groups statistically not significant, there is no real basis for making inferences regarding apparent trends.

Table 21: Thirty-Day Major Clinical Events – ASSENT I

	30 mg	40 mg	50 mg	Total
N	1705	1457	73	3235
death	117 (6.9%)	88 (6.0%)	3 (4.1%)	208 (6.4%)
death or non-fatal stroke	133 (7.8%)	104 (7.1%)	3 (4.1%)	240 (7.4%)
recurrent MI	140 (8.2%)	86 (5.9%)	4 (5.5%)	230 (7.1%)
pulmonary edema	73 (4.3%)	78 (5.4%)	5 (6.8%)	156 (4.8%)
cardiogenic shock	62 (3.6%)	55 (3.8%)	3 (4.1%)	120 (3.7%)
cardiac revascularization	503 (29.5%)	408 (28.0%)	27 (37.0%)	938 (29.0%)
anaphylaxis	1 (0.1%)	3 (0.2%)	0	4 (0.1%)

Bleeding

Serious bleeding events, non-serious bleeding events and transfusions are summarized in Table 22. The incidence of serious bleeding events appears similar in the 30 and 40 mg groups, albeit

Table 22: Selected Serious Bleeding Events, Non-Serious Bleeding Events, and Transfusions – ASSENT I

	30 mg	40 mg	50 mg	Total
N	1705	1457	73	3235
Numbers of subjects with at least one Serious Bleed	48 (2.8%)	39 (2.7%)	3 (4.1%)	90 (2.8%)
Retroperitoneal hemorrhage	2 (0.1%)	0	1 (1.4%)	3 (0.1%)
Catheter-site hemorrhage	4 (0.2%)	4 (0.3%)	1 (1.4%)	9 (0.3%)
ICH	16 (0.9%)	11 (0.8%)	0	27 (0.8%)
GI hemorrhage	8 (0.5%)	5 (0.3%)	0	13 (0.4%)
Numbers of subjects with at least one Non-serious Bleed	536 (31.4%)	492 (33.8%)	35 (47.9%)	1063 (32.9%)
Injection-site hemorrhage	43 (2.5%)	37 (2.5%)	4 (5.5%)	84 (2.6%)
Catheter-site hemorrhage	168 (9.9%)	150 (10.3%)	15 (20.5%)	333 (10.3%)
Epistaxis	43 (2.5%)	40 (2.7%)	4 (5.5%)	87 (2.7%)
Hematuria	100 (5.9%)	103 (7.1%)	8 (11.0%)	211 (6.5%)
Received Blood Transfusion	72 (4.2%)	66 (4.5%)	4 (5.5%)	142 (4.4%)

the 40 mg group was enrolled after the implementation of protocol changes designed to reduce bleeding; the 50 mg group was enrolled almost exclusively before the protocol changes. Thus, the groups are not directly comparable.

Reviewer's Comment(s): To address this issue, CBER assessed the rate of serious bleeding events in subjects in the 30 mg group treated *after* the 9/96 protocol changes were implemented. The rate of serious bleeding events was 2.8% (42/1475), the same rate as observed for the entire 30 mg treatment group, and essentially the same rate observed in the 40 mg group (2.7%). Thus, for the contemporaneously randomized 30 and 40 mg groups, there appears to be no difference in rates of serious bleeding events.

Non-serious bleeding events and the incidence of transfusion appear to increase with increasing dose; however, the limitations noted above apply.

Support for Weight-Adjusted TNK Dosing

Exploratory analyses were conducted to evaluate the relations between dose, weight and weight-adjusted dose with mortality, ICH, and serious bleeding events. The data supported weight-adjusted TNK dosing on the basis of a demonstration of a relation between serious bleeding events and increasing dose/weight. The results were not conclusive for mortality or ICH; the strength of the data was limited by several factors:

- Only ~2% of subjects were randomized to the 50 mg dose. With 98% of subjects receiving 30 or 40 mg TNK, the range of dose/weight was limited.
- The 30 and 40 mg groups are not directly comparable with respect to safety because they were differentially affected by protocol changes made explicitly to enhance safety. Specifically, all subjects in the 40 mg group were enrolled after protocol amendment, whereas 85% of subjects in the 30 mg group were enrolled after protocol amendment.
- Weight was not recorded in 7.9% of subjects. Although missing data were equally distributed between the 30 and 40 mg dose groups, there were disproportionate numbers of missing weights in subjects who died or experienced ICH.
- Only a small number of ICH events (25) were recorded, and weights were recorded in only 18 of these subjects, limiting the usefulness of the data.

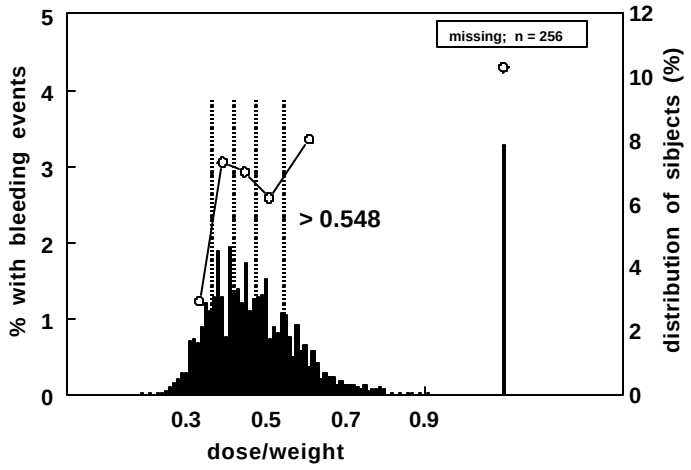
The results of the sponsor's analyses are summarized below:

Mortality: The sponsor's exploratory analyses showed that log[dose/weight] was directionally associated with mortality (i.e., increasing mortality with increasing log[dose/weight]). However, in ASSENT I (and as generally observed in other thrombolytic trials), there was a highly statistically significant negative relation between weight and mortality (increasing mortality with decreasing weight). Using the terms log[dose/weight] and log[weight] in a logistic regression model, there was no significant relation between weight-adjusted dose and mortality (p=0.57). (The extent to which the data were confounded by the factors noted above can not be determined.)

ICH: There was no consistent pattern with respect to ICH and dose or weight-adjusted dose, not surprising considering that there were only 18 ICH events in subjects with recorded weights. The sample size calculations did not take into consideration the performance of exploratory analyses based on subject weight, and did not address the possibility that weights would not be assessed in a substantial fraction of subjects with ICH.

Serious Bleeding Events: Serious bleeding events occurred more frequently than ICH or death and were less confounded by missing data. These data provided the only real support for weight-adjusted TNK dosing as a means to enhance safety, and are summarized in Figure 7. The line graph (top) refers to the left Y-axis and shows % of subjects with severe bleeding events. The bottom histogram refers to the right Y-axis and shows the distribution of weight-adjusted doses administered. There is a trend towards increased serious bleeding events in the upper dose/weight quintile (weight-adjusted dose > 0.548 mg/kg).

Figure 7: Serious Bleeding by mg/kg Quintile



Reviewer's Comment(s): Details of the statistical assessment of the relation between serious bleeding, dose, weight and weight-adjusted dose were not originally provided in the license application, but were subsequently provided by the sponsor on April 26, 2000 at CBER's request. Using logistic regression, with serious bleeding events as the dependent variable, there is a significant relation between log[dose/weight] and serious bleeding, with increasing log[dose/weight] associated with an increased incidence of bleeding events. There is also a significant negative relation between serious bleeding and log[weight]. With log[dose/weight] and log[weight] evaluated jointly, however, there is no significant relation between serious bleeding events and log[dose/weight] or log[weight]. Although the p-value of the overall model was 0.017, the p-value for log[dose/weight] was 0.75. [redacted]

Non-Bleeding Adverse Events

Twenty-six percent (26%) of subjects reported at least one serious non-bleeding adverse event. Virtually all relate to cardiovascular or pulmonary systems, and are typical for an AMI patient population. Non-serious non-bleeding adverse events were reported in 82% of subjects, and were also typical of an AMI patient population. The most common non-cardiovascular adverse events were nausea, headache, back pain, pain and dyspepsia – all observed frequently in this population. Fever was reported in 9.6% of subjects. Again, fever is commonly observed in the setting of AMI.

Laboratory Values

Laboratory values were reported at baseline, 24 – 48 hours, and discharge. On inspection, the changes in the treatment groups in ASSENT I appear typical for a hospitalized AMI patient population, and similar in the three treatment groups. The informativeness of these data is somewhat limited, however, because of the patient population (see below), and because there is no non-TNK control group in ASSENT I.

Reviewer's Comment(s): The numbers of subjects with reported values vary widely and are frequently incomplete, depending on the particular parameter assessed and the time of the measurement. For example, serum lactate dehydrogenase (LDH) is reported for only 17 subjects (< 1%) at 24 – 48 hours, and for only 59% of subjects at discharge, whereas serum sodium is reported for 93% of subjects at 24 –

48 hours and 79% of subjects at discharge. The completeness of assessment for other laboratory parameters fall between these extremes.

Serial changes in a number of laboratory values are typically observed in the AMI patient population. For example, it is not unusual for AMI patients to present with leukocytosis and mild hyperglycemia (both may be related to catecholamine release), such that decreases in leukocyte count and glucose are expected during the course of hospitalization. Increases in hepatic transaminases and lactate dehydrogenase (LDH) are expected after AMI, with return towards normal over the course of hours to days. Moreover, during the course of hospitalization for AMI, phlebotomy is performed frequently to assess aPTT and other laboratory values. Decreases in hemoglobin and hematocrit during hospitalization due to phlebotomy are not unusual. When the reported changes in laboratory values are considered in the context of changes typical of an AMI patient population, there are no unusual safety signals apparent.

ASSENT I Summary

In this 3235-subject study of 30, 40, and 50 mg TNK in AMI, the overall safety profile appears to be generally comparable to accelerated t-PA based on historical data from GUSTO I. The ICH rate was 0.9% (95% CI: 0.5%, 1.5%) for the 30 mg group, 0.6% (0.3%, 1.2%) for the 40 mg group, and 0 (0, 4.9%) for the limited number of subjects (73) in the 50 mg group. These rates were not dissimilar to the ICH rate for t-PA in GUSTO I (0.7%). Thirty-day mortality rates (6.9% for the 30 mg group and 6.0% for the 40 mg group) were also comparable to those of recent clinical trials of thrombolytic agents in AMI.

ASSENT I was designed with the intent of assessing the relative safety of two TNK doses (30 and 50 mg), thought to be at the margins of the range of doses expected to be effective in achieving clot lysis, based on phase 1 data from TIMI 10A. Due to safety considerations, however, the 50 mg dose was replaced with a 40 mg dose, when enrollment was only 10% complete. Thus, the study primarily assessed the safety of 30 and 40 mg TNK doses. The study does not demonstrate clear safety differences between the groups, perhaps in part due to the limited range of difference between the 30 and 40 mg doses. The sponsor performed exploratory analyses on the relation between weight-adjusted dose and safety outcomes. The analysis of severe bleed events provided minimal to moderate support for weight-adjusted dosing in the phase 3 study.

Protocol N0747g – ASSENT II

Title:	A Phase III, Randomized, Double-Blind, Parallel-Group, International Trial of a Single Bolus of TNK-Tissue Plasminogen Activator (TNK) Versus an Accelerated Infusion of rt-PA (Alteplase, T-PA) in Acute Myocardial Infarction: ASSENT II (Assessment of the Safety and Efficacy of a New Thrombolytic Agent)
Study Period:	October 1997 to December 1998
Funding:	Boehringer Ingelheim GbmH, Germany; Genentech, Inc., USA
Size/Scope:	17,005 subjects enrolled at 1,022 sites in 29 countries in North America, South America, Europe, Africa, and Australia

Study Background

Rationale For Phase 3 Dose Selection

The angiographic and safety data from TIMI 10B and ASSENT I provided the framework for the TNK dose regimen in ASSENT II. Exploratory analyses in TIMI 10B suggested improved thrombolysis with increasing weight-adjusted TNK dose, with a plateau in the range of 0.5 mg/kg to 0.6 mg/kg. In ASSENT I, risk of serious bleeding events increased with increasing standardized dose and decreasing weight. Considered together, the sponsor selected 0.53 mg/kg a the target proportionality for TNK dosing, and noted: "*Based on practical considerations for single bolus injection, five dosing tiers were used in ASSENT II as the optimal body weight adjustment dosing scheme for TNK-tPA*."

Reviewer's Comment(s): It is true that "single bolus injection" is a laudable goal; however, this would have been accomplished using a straightforward 0.53 mg/kg dosing paradigm, without a stepped approach.

Table 23: TNK dosing in ASSENT II

subject weight (kg)	target dose (mg) for 0.53 mg/kg	TNK dose (mg)	volume reconstituted TNK (mL)	actual dose range (mg/kg)
< 60	< 31.8	30	6	> 0.5
≥ 60 and < 70	31.8 to 37.1	35	7	> 0.5 to 0.58
≥ 70 and < 80	37.1 to 42.4	40	8	> 0.5 to 0.57
≥ 80 and < 90	42.4 to 47.7	45	9	> 0.5 to 0.56
≥ 90	≥ 47.7	50	10	≤ 0.56

No clear rationale was provided for the stepped scheme, per se. Thus, the efficacy and safety of TNK, with particular reference to the stepped dosing paradigm, will be considered in this review.

The five TNK dosing tiers used in ASSENT II and included in the proposed labeling are summarized in Table 23. Also shown are the actual ranges of weight-adjusted doses for each dose.

Objectives

Primary

To demonstrate efficacy of TNK as determined by no unacceptable inferiority in 30-day mortality between bolus TNK and accelerated infusion of t-PA

Secondary

To compare the in-hospital rates of:

- death
- stroke
- intracranial hemorrhage (ICH)
- major bleeding (other than ICH, requiring transfusion of blood but not with hemodynamic compromise requiring intervention)
- non-fatal major cardiac events including: myocardial reinfarction, heart failure, major arrhythmias, invasive procedures

- other major clinical events including: sustained hypotension, acute mitral regurgitation, acute ventricular septal defect, pericarditis, pulmonary embolism and tamponade
- to compare the net clinical benefit, defined as the absence of 30-day mortality and in-hospital stroke.
- to compare 30-day mortality, stroke rates, and "net clinical benefit" (≡ death or non-fatal stroke) according to age (≤ 75 versus > 75), time to treatment (0 – 2 hrs, 2 – 4 hrs, 4 – 6 hours), infarct location (anterior versus non anterior) and history of previous MI (y/n)

Study Design

This was to be an international, multicenter, randomized (1:1), double-blind, double-dummy, controlled, parallel-group study in 16,500 patients with AMI presenting within 6 hours of symptom onset, treated with either a bolus of TNK or an accelerated infusion of t-PA. Boehringer Ingelheim had primary responsibility for non-North American sites; Genentech had primary responsibility for the North American sites.

Steering Committee

A Steering Committee, composed of the chairman and members from all participating countries, was to provide scientific direction for the study, and to meet periodically to assess the study's progress and address policy issues. The Committee chairman was responsible for communicating with the DSMB and sponsor. Boehringer Ingelheim and Genentech representatives attended all meetings as non-voting members.

Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) comprised of independent cardiologists and an independent biostatistician was to conduct a review of the safety data. The DSMB was also to conduct analyses of the data at the request of the Steering Committee and/or if the lower confidence interval for the ICH rate were ≥ 1.0% in one of the treatment groups.

Stroke Review Panel

A Stroke Review Panel, consisting of independent neurologists and neuroradiologists, reviewed all stroke events and classified stroke events as primary hemorrhagic or ischemic.

Leuven Coordinating Center

The ASSENT II Coordinating Center (LCC), located at the University Medical Center in Leuven, provided overall study coordination and was supported by the Duke Clinical Research Institute in North Carolina, which provided coordination services for North America.

Definitions of Terms

Bleeding Classification

Major bleeding was classified as moderate or severe/life-threatening as follows:

Moderate bleeding ≡ a requirement for blood transfusion, in the absence of hemodynamic compromise requiring intervention.

Severe/life-threatening bleeding ≡ causing hemodynamic compromise requiring intervention (blood or fluid replacement, inotropic support, LV assist device, surgical repair) or resulting in death

Reinfarction

- Recurrent MI within the first 18 hours of study agent:

≡ recurrent signs and symptoms of ischemia at rest accompanied by new or recurrent ST elevation ≥ 0.1 mV in at least 2 contiguous leads lasting ≥ 30 minutes

- Recurrent MI after 18 hours:

≡ new Q waves in two or more leads and/or CPK evidence of AMI, as follows: re-elevation of CK-MB > ULN and increased by ≥ 50% over the previous value. If CK-MB not available, the total CK must either be re-elevated to ≥ 2X ULN and increased by ≥ 25% or to ≥ 200 U/mL over the previous value; if re-elevated to < 2X ULN, the total CK must exceed the upper limit of normal by ≥ 50% and exceed the previous value by 2-fold or to ≥ 200 U/mL.

- Recurrent MI after PTCA (± stent):

≡ CK-MB (or CK, if MB is not available) > 2X ULN and ≥ 50% greater than the previous value and/or new Q waves in two or more contiguous leads

- Recurrent AMI after CABG surgery:

≡ CK-MB (or CK, if MB is not available) of > 5X ULN and ≥ 50% greater than the previous value and/or new Q waves in two or more contiguous leads

Recurrent Myocardial Ischemia

≡ recurrent anginal symptoms at rest accompanied by new ST-T changes in ≥ 2 contiguous leads without enzyme elevations

Heart Failure

≡ Killip Class II or IV after start of study medication (the definition was Killip III and IV in the original protocol, but was extended to include Killip II per a September 29, 1997 clarification letter in response to CBER's comments).

Major Arrhythmias

≡ sustained ventricular tachycardia (VT) (VT lasting ≥ 30 seconds or causing symptoms/hypotension or requiring cardioversion), ventricular fibrillation, asystole, atrial fibrillation, second and third degree atrioventricular block

Invasive Procedures

≡ CABG, PTCA, stent deployment, intra-aortic balloon

Other Major Cardiac Events

≡ sustained hypotension, acute mitral regurgitation, acute ventricular septal defect (VSD), pericarditis, pulmonary embolism and tamponade

Patient Population

The intended patient population was subjects with AMI who presented within 6 hours after symptom onset.

Reviewer's Comment(s): The requirement for treatment within 6 hours of symptom onset differs from the phase 1 and 2 studies, but is consistent with other larger trials of thrombolytic agents in AMI.

Inclusion Criteria

- onset of symptoms of AMI within 6 hours of randomization
- ST-segment elevation ≥ 0.1 mV in two or more limb leads or > 0.2 mV in two or more contiguous precordial leads indicative of AMI, or left bundle-branch block
- age ≥18

Exclusion Criteria

- hypertension ≡ BP > 180/110 mmHg (BPs > 180 mmHg and/or BPd > 110 mmHg) on repeated measurements during current admission prior to randomization
- use of Abciximab or other marketed GPIIb/IIIa antagonists within the preceding 12 hours
- major surgery, biopsy of a parenchymal organ, or significant trauma within 2 months
- minor head trauma and any other trauma occurring after onset of the current myocardial infarction
- known history of stroke or transient ischemic attack or dementia
- known structural damage of the central nervous system
- current therapeutic oral anticoagulation with International Normalized Ratio (INR) >2
- prolonged cardiopulmonary resuscitation (>10 minutes) in the previous 2 weeks
- pregnancy or lactation, parturition within the previous 30 days. Women of childbearing potential must have a negative pregnancy test.
- known participation in another investigative drug study or device protocol within 30 days

Treatment

Material Source

TNK and t-PA were provided as sterile, lyophilized powders for reconstitution with Sterile Water for Injection, USP (or EP for non–North American sites). For the double-dummy study design, each subject was assigned a treatment box containing one of the following: 1) TNK with a placebo in place of t-PA; or 2) t-PA with placebo in place of TNK.

TNK (or corresponding placebo) was supplied in a 50 mg vial with a 20 mL vial of diluent (SWFI). TNK contained the following excipients: arginine, phosphoric acid, and polysorbate 20. Only 10 mL of the 20 mL of diluent was to be used, providing a TNK solution concentration of 5 mg/mL.

[redacted]

t-PA was supplied in two-50 mg vials of Activase® (Genentech) t-PA and two 50 mL vials of SWFI for reconstitution, for a concentration of 1 mg/mL. The t-PA contained the following

excipients: arginine, phosphoric acid, and polysorbate 80. Only Genentech Activase® was used in this study. [redacted]

Dose and Administration

TNK (or corresponding placebo) was to be given *first* by the IV route over 5-10 seconds (into an IV line of normal saline, as close as possible to the insertion site). The TNK dose was summarized in Table 23.

t-PA (or corresponding placebo) was to be given *after* TNK (or its placebo) A maximum of 100 mg t-PA® was administered as an accelerated 90-minute infusion regimen. Subjects were to receive a 15 mg initial IV bolus, followed by 0.75 mg/kg administered over 30 minutes (not to exceed 50 mg), followed by 0.50 mg/kg administered over the next 60 minutes (not to exceed 35 mg). Infusion was to be by infusion pump into a peripheral IV line of normal saline.

Randomization

The randomization schedule was to be established by the Data Center and held solely by the [redacted]Clinical Safety Officers of Boehringer Ingelheim, and the randomization service. Randomization was to be stratified by center and blocked within centers. Treatment assigned by telephone randomization via IVRS. Patient study numbers were to be the same as the drug kit number actually used. Subjects were considered as "randomized" when drug kit number was assigned, regardless of whether the drug kit number was confirmed or if the drug kit was used. All subjects randomized or who received study medication without randomization were considered "enrolled."

Concomitant Medications

Specially Directed:

- Aspirin, non-enteric-coated, 150 – 325 mg orally immediately upon study entry, then 150 – 325 mg once daily. Alternatively, 100 – 250 mg aspirin given intravenously was acceptable if subjects unable to ingest orally. Subjects who had taken aspirin within 12 hours prior to randomization were to start aspirin the following day.
- Heparin was to be administered as soon as possible after study entry. For subjects ≤ 67 kg, 4000 units IV bolus, then 800 units/hours infusion; for subjects > 67 kg, 5000 units IV bolus, then 100 units/hour for 48-72 hours. aPTT was performed at baseline, 6, 12, 24 and 48 hours after the start of study agent, and daily thereafter while the subject was on IV heparin. The infusion was adjusted to maintain aPTT at 50 – 75 seconds, and aPTT was assessed 6 hours after adjustments in infusion rate. No additional heparin was to be given during diagnostic catheterization while subjects were receiving IV heparin. For rescue angioplasty, the target activated clotting time (ACT) was given as 300 seconds. For rescue coronary intervention in which abciximab was utilized, the target ACT was 200 seconds. Additional heparin was to be administered in increments no larger than 2500 units.

Prohibited: low molecular weight heparin (LMWH) during IV heparin infusion

Discouraged: abciximab during the first 24 hours after randomization

Discretionary: β-adrenergic blocking agents; calcium antagonists

Interventions

Cardiac catheterization and revascularization (PTCA ± stenting, or CABG) were permitted at the discretion of the treating physician, and recorded on the appropriate page of the case report form. For percutaneous interventions performed during the first 24 hours, large doses of additional heparin were to be avoided (see above). Treatment with ticlopidine following stent deployment was allowed.

Monitoring

A flow chart of evaluations is shown in Table 24. Additional serial 12-lead ECGs and cardiac enzymes (CK, CK-MB) were to be obtained for recurrent signs or symptoms of myocardial ischemia or recurrent AMI.

Table 24: ASSENT II Study Flow Chart

	baseline	in-hospital	discharge
medical history	X		
physical examination	X		X
demographic data	X		
hemoglobin / hematocrit	X	anytime	
CK/CK-MB	X	anytime	
pregnancy test (if applicable)	X		
ECG	X	@ 24 - 36 hours	X

Hemoglobin and hematocrit were to be determined for bleeding event(s). Pre- and post-transfusion hemoglobin and hematocrit were to be assessed if blood was transfused. In-hospital nadir hemoglobin and hematocrit values were to be recorded in the CRFs.

The in-hospital phase of the study was defined as from randomization through hospital discharge, the latter defined as end of hospitalization at either the hospital where a subject was randomized, or the hospital to which a subject was routinely transferred for continued treatment of the initial event. Subjects experiencing a new neurologic deficit at any time after randomization were to have immediate (within 24 hours) computed tomographic (CT) scanning (preferred) or magnetic resonance imaging (acceptable) to assess the possible occurrence of ICH. In addition, all subjects were to have seen by a neurologist to assist with the completion of the stroke event form. Clinical information pertinent to neurologic events (copies of cranial imaging studies) was to be submitted for blinded review and classification by the Stroke Review Committee.

Thirty-Day Follow-Up

Subjects were to be contacted by telephone (or return to the hospital) for follow-up at 30 – 37 days. Vital status and clinical outcome were to be determined for all subjects and reported on the appropriate CRF pages. For subjects who could not be contacted, follow-up could be with the subject's family or physician. Similar methods were to be used for 1-year (365-395 day) vital status.

Criteria for stopping study treatment

TNK: Not applicable (one-time bolus administration)
t-PA: In case of suspicion of ICH or significant bleeding, the infusion was to be stopped immediately. Appropriate measures were to be at the discretion of the physician.

Response Variables

Primary Endpoint

The prospectively-defined primary efficacy endpoint was 30-day mortality (see statistical plan for details, page 55).

Secondary Endpoints

- Exploratory analyses on the 30-day mortality endpoint (see statistical plan for details, below).
- "Net clinical benefit," defined as the absence of mortality and non-fatal stroke at 30 days
- Rate of non-fatal cardiac in-hospital events (recurrent MI, sustained hypotension, occurrence of pulmonary edema and/or cardiogenic shock, major arrhythmias, invasive cardiac procedures (PTCA, stent placement, CABG surgery, IABP), pericarditis, acute mitral regurgitation, pulmonary embolism, and tamponade)
- Thirty-day mortality and net clinical benefit at 30 days according to age, time to treatment, infarct location, and history of previous MI

Safety Objectives

- stroke, ICH, major bleeding (other than ICH), serious and non-serious adverse events
- stroke and ICH rates according to age and time to treatment
- The protocol specified that the primary safety analyses were to be based on the intent-to-treat population.

Statistical Analysis Plan

CBER initially advised the sponsor that the satisfactory demonstration of TNK's efficacy would require a randomized study design with a well-characterized established agent as the active comparator and mortality as the efficacy endpoint.

Sufficiency Criteria

The sponsor was advised that interpretation of the primary efficacy results would be based upon a comparison of the 95% one-sided CI for the relative risk (RR) of mortality of TNK relative to the active comparator, to a limiting value termed the "Sufficiency Criterion." This term represents the smallest amount of diminished efficacy that must be excluded compared to the active control agent, i.e., the minimum acceptable preserved benefit of the new agent compared to active control agent.

I. TNK Versus a "Generic" Thrombolytic Agent

The original approach to the Sufficiency Criterion was based on the premise that all thrombolytic agents are essentially similar in efficacy. The Fibrinolytic Therapy Trialists' (FTT) Collaborative Group meta-analysis, a meta-analysis of several placebo-controlled studies involving a variety of thrombolytic agents, was deemed to provide an acceptable estimate of efficacy of a "generic" or typical thrombolytic agent versus placebo (*Lancet* 1994;343:311). Expressing the efficacy of the active comparator as the 95% one-sided CI bound on the RR of mortality ("generic" active agent versus placebo), the Sufficiency Criterion involved a "50% Rule." This rule stipulated that 50% of the estimate of risk reduction of the active comparator versus placebo would define the limit of acceptability for excess risk of the new agent versus active comparator. Using this approach, evidence of sufficient retained efficacy of a new thrombolytic agent would require that the 95%

one-sided CI of the RR of mortality (new agent versus active comparator) excludes a value of 1.122.

II. TNK Versus Accelerated t-PA – A Specific Thrombolytic Agent/Regimen

Based on the postulate that efficacy may not be the same for all thrombolytic agents (or for all regimens of a specific agent), a different but related approach was provided for assessment of the adequacy of retained efficacy of a new thrombolytic agent. For this approach, the calculation of a Sufficiency Criterion was based on the estimated efficacy of the specific active comparator agent/ regimen versus placebo, in this case, accelerated ("90 minute" or "front loaded") t-PA versus placebo. With respect to accelerated t-PA, however, an adequate body of clinical study information comparing it to placebo was lacking; therefore, its efficacy relative to placebo had to be estimated through the use of an intermediary agent.

Specifically, the GUSTO I study provided clinical data comparing accelerated t-PA to a regimen of streptokinase (SK), and the efficacy of SK could be estimated from placebo-controlled trials of SK. An approach linking the efficacy of accelerated t-PA versus SK to that of SK versus placebo was used to provide an estimate of efficacy of accelerated t-PA versus placebo. Given that this approach began with the premise that all thrombolytic agents are not equivalent in efficacy, data from the FTT data meta-analysis were not appropriate for use, because an estimate of efficacy of a typical or "generic" thrombolytic agent was counter to the initial postulate.

CBER performed a meta-analysis of four studies of SK versus placebo (ISIS-2, GISSI-1, ISAM and EMERAS) to form an estimate of the RR and 95% CI of mortality of SK versus placebo. The meta-analysis was performed in such a way as to weight each study for its relative size, and only subjects treated within 6 hours of symptom onset were included in the analysis, to provide results that could be generalized to the patient population of ASSENT II. With this approach, a high degree of confidence was given to the derived estimate of minimum expected efficacy of SK versus placebo, because the analysis included a large number of subjects enrolled in several studies utilizing disparate treatment protocols and investigators.

Conversely, the estimate of efficacy of accelerated t-PA versus SK was based on a single open-label study. Thus, the basis for estimation of RR was deemed to be weaker than that for SK versus placebo. In keeping with the Agency position that efficacy evidence from a single trial should be statistically persuasive (i.e., a very low p-value), FDA applied a more conservative approach to the estimation of RR from the single GUSTO I trial, using a 99.5% CI for these data in lieu of the 95% CI.

The calculation of the estimate of the expected efficacy of accelerated t-PA versus placebo was based on an approach involving the application of the CIs to the variances of the SK meta-analysis and GUSTO I. The complex CI limit on RR for accelerated t-PA versus placebo was estimated to be 0.7777. Applying the "50% rule" of minimum retained risk reduction yielded a value of 0.8888 [1 – ½X (1 – 0.7777)] as the minimum acceptable efficacy of TNK versus placebo. Thus, the ratio of these two RRs ([TNK versus placebo] / [t-PA versus placebo] = 0.8888/0.7777) provided the maximum acceptable RR of TNK versus accelerated t-PA, a Sufficiency Criterion of 1.143.

This criterion was less conservative than the criterion calculated from the FTT meta-analysis for a "generic" thrombolytic, and became the accepted Sufficiency Criterion for ASSENT II. Thus, a study result wherein the 95% one-sided CI of the RR of mortality of TNK versus t-PA excluded a

value of 1.143 was deemed to provide evidence that TNK retained sufficient efficacy to be supportive of a marketing application.

Study Populations

The sponsor defined 3 subject populations for analyses:

1. Intent-to-treat population: all randomized subjects (primary analysis)
2. As-treated population: all treated subjects; actual study drug given (sensitivity analysis)
3. Randomized and treated population: all subjects who were randomized and treated; actual study drug given (sensitivity analysis)

Primary Endpoint

The primary efficacy endpoint was 30-day mortality, defined as death at or before 30 days after randomization/enrollment. The endpoint was analyzed using the following methods:

- nonparametric covariate adjusted method, which served as the primary analysis. Five prespecified variables (age [continuous], Killip class [discrete], heart rate [continuous], systolic blood pressure [continuous], and infarct location [discrete]) were included in the analysis
- unadjusted method, which served as a supportive analysis
- A semiparametric covariate adjusted method (logistic regression) served as an exploratory analysis. The 5 prespecified covariates and interactions of the treatment with the covariates were examined in the analysis. In addition, an exploratory analysis to adjust for potentially imbalanced baseline variables was planned.

Agreements reached between Genentech and CBER on the interpretation of the endpoint were formalized within IND, and were never written into the protocol. On the basis of these agreements, a Sufficiency Criterion of 1.143 was regarded as the maximum acceptable RR of TNK versus accelerated t-PA to establish non-inferiority.

Secondary Efficacy Endpoints

- “Net Clinical Benefit” (defined as death or nonfatal stroke at 30 days)
- Rates of the following major non-fatal in-hospital cardiac events:
  - cardiogenic shock and/or pulmonary edema
  - recurrent MI
  - sustained hypotension
  - major arrhythmias
  - invasive cardiac procedures (PTCA, stent placement, CABG surgery, IABP)
  - pericarditis
  - acute mitral regurgitation
  - pulmonary embolism
  - tamponade
  - acute ventricular septal defect (VSD)

- Rates of 30-day mortality and net clinical benefit by age (≤ 75 years vs. > 75 years), time to treatment (0–2 hours, 2–4 hours, 4–6 hrs), infarct location (anterior versus non-anterior) and history of previous MI (yes versus no).

For net clinical benefit, the methods described for the primary efficacy endpoint were also applied to estimate the adjusted absolute difference of the event rates and its one-sided 95% CI as well as the adjusted relative risk of the event rates and its one-sided 95% CI. Exploratory analyses were performed to adjust for potentially imbalanced baseline variables for these secondary endpoints and to increase the precision of the estimates.

The remainder of the secondary endpoints (e.g., clinical outcomes) were used to assess potential treatment differences. Two-sided (asymptotic) 95% CIs were calculated, and p-values of Fisher's exact test were presented.

Exploratory Variables

Exploratory variables included the following:

- Death or nonfatal ICH, death or nonfatal, disabling stroke, death or nonfatal, disabling ICH, recurrent angina, anaphylaxis, TIA, acute VSD, Killip class at discharge

The Fisher's exact test was used to assess potential treatment differences. Two-sided 95% CIs were calculated, and p-values were presented.

Safety Endpoints

The unadjusted RR of the TNK and t-PA groups with two-sided 95% CIs and p-values were presented for: primary ICH, total stroke and ischemic stroke. The Fisher's exact test was used to assess potential treatment differences, and p-values were presented for: total bleeding, major bleeding and units of blood transfused. All other adverse events were tabulated, and no p-values were specified.

Planned Subgroup Analyses

Efficacy

Thirty-day mortality and the composite of death or non-fatal stroke ("net clinical benefit") were compared between treatment groups using the subgroups shown in Table 25.

In each subgroup, the relative risk of 30-day mortality and net clinical benefit and the two-sided 95% CI was estimated. For each covariate of the subgroup analyses, a logistic regression, with the interaction term treatment X covariate included, was used as an exploratory analysis.

Safety

Primary ICH, total stroke, and ischemic stroke were compared between treatment groups for the subgroups including age (≤/> 75), time to treatment (0-2, 2-4, 4-6 hours), gender, systolic BP and weight. In each subgroup the relative risk for primary ICH, total stroke, and ischemic stroke and the two-sided 95% CIs were estimated using logistic regression.

Table 25: Efficacy Endpoints – Subgroup Analyses

characteristic	subgroups
age	<= 75 years; > 75 years
time to treatment	0-2 hr; > 2-4 hr; > 4 hr
infarct location	anterior vs. other
previous MI	Y/N
sex	M/F
heart rate	<60, 60-79; 80-99;100+
systolic blood pressure	<100 mmHg; 100-139 mmHg; 140-174 mmHg; >= 175 mmHg
Killip Class	I; II; III; IV
hypertension	Y/N
diabetes	Y/N
prior CABG	Y/N
prior PTCA	Y/N
EU vs. non-EU	
US vs. non-US	
race	Caucasian; African descent; Asian; other
weight (1)	< 67 kg; >= 67 kg
weight (2)	< 60 kg, 60-69 kg; 70-79 kg; 80-89 kg; >= 90 kg

Concomitant Medications

Concomitant medication use during hospitalization was recorded in the CRFs. The percentage of subjects was calculated with respect to the number of subjects with non-missing information for that particular item. The Fisher's exact test was used to assess potential treatment differences; p-values were presented.

Study Performance

Subjects were enrolled from October 1997 to December 1998. The database was locked on March 31, 1999.

Changes to Protocol

There were two clarification letters and one protocol amendment, the latter dated April 23, 1998.

Clarification Letters

An initial clarification letter was issued September 29, 1997. Its chief purpose was to reorder the primary and secondary efficacy and safety objectives and to define net clinical benefit as absence of mortality and non-fatal stroke at Day 30 (instead of disabling stroke at Day 30). A second clarification letter was issued February 12, 1998, to promote consistency in adverse event reporting. As a consequence of this, a Study Procedure Clarification dated February 13, 1998 was issued in parallel to clarify the monitoring procedures.

Protocol Modifications and Amendment

A protocol amendment summarizing all changes, minor revisions and clarifications from the clarification letters was issued on April 23, 1998. The revision primarily addressed changes in the statistical analytic plan resulting from discussions with CBER. Other changes were mostly

procedural, without a material effect on study conduct. The study design summarized in this document (above) reflects the *revised* protocol.

ASSENT II Study Results

Between October 1997 and December 1998, 17,005 subjects were enrolled at 1,022 sites in 29 countries. Of these subjects, 16,949 (99.67%) were randomized and comprise the intent-to-treat (ITT) population; 56 subjects (0.33%) were not randomized. For both treatment groups, ~97% of subjects received the thrombolytic agent assigned by randomization, 0.36% received the wrong agent, and 2.63% received no agent (Table 26):

Table 26: Intent-to-Treat Study Population – Actual Treatment Received

	TNK N (%)	t-PA N (%)	total N (%)
Randomized (intent-to-treat)	8461	8488	16949
received assigned study agent	8198 (96.89)	8245 (97.14)	16443 (97.01)
received wrong study agent	27 (0.32)	34 (0.40)	61 (0.36)
received no study agent:	236 (2.79)	209 (2.46)	445 (2.63)
adverse event (including death) before study agent	10 (0.12)	13 (0.15)	23 (0.14)
exclusion criteria found	76 (0.90)	59 (0.70)	135 (0.80)
technical reasons; unknown or missing reasons	150 (1.77)	137 (1.61)	287 (1.69)

Among the 445 randomized subjects who received no study agent, 88 (~20%) underwent PTCA within the first study day (PTCA day code = 0 or 1): 45 subjects (19.1%) in the TNK group and 43 subjects (20.6%) in the t-PA group.

Reviewer's Comment(s): Primary PTCA, and more recently, PTCA accompanied by stent implantation, have been gaining popularity as treatment strategies for AMI. In the present study, the performance of primary PTCA in a substantial fraction of selected subjects would have had the potential to limit the generalizability of the study results. Fortunately, for the study as a whole, PTCA was reported in only ~0.5% of subjects overall, and its use was balanced between treatment arms.

CBER also assessed the frequency of PTCA (without thrombolytic agent) by site, and found no sites with an apparent tendency to utilize primary PTCA in favor of the blinded thrombolytic agent. In the TNK group, there were 7 sites with 2 such subjects per site; in the t-PA group, there were 3 subjects at a single site. No site reported > 3 subjects who failed to receive the thrombolytic agent and underwent PTCA within the first day.

Protocol Violations

The sponsor reported that 297 subjects in the intent-to-treatment population were identified by investigators as ineligible. Through review, 664 additional subjects were found to have violated inclusion or exclusion criteria. These subjects were allocated evenly between TNK and t-PA groups. In both treatment groups, approximately 70% of these subjects received their assigned

thrombolytic agent. Treatment > 6 hours after symptom onset was balanced between groups and accounted for 475 violations (~72% of all violations, and in 2.8% of subjects overall). Other principal reasons for subject ineligibility were known history of stroke, transient ischemic attack, or dementia.

Reviewer's Comment(s): CBER's review of the database found 19 subjects in each treatment group reported as treated > 24 hours after symptom onset. For a minority of these subjects (and for other subjects with treatment reported as initiated > 6 hours after symptom onset), it appeared that investigators recorded the time of symptom onset or treatment bolus in error (i.e., symptom onset at entered as midnight, interpreted as the beginning of a day (00:00:01) when the investigator's intention appeared to be the end of the day (23:59:59). Occasionally, a date appeared to be entered incorrectly. It is also possible that a fraction of subjects exhibited a stuttering symptom course leading to AMI, with an ambiguous time of symptom onset. In any case, these irregularities in a small subset of subjects are unlikely to have had a meaningful effect on the data set overall.

An inherent assumption in a non-inferiority study design is that the active comparator agent is efficacious in the patient population studied. For the present study, this assumption is contingent on verification of the diagnosis of AMI in study participants. Because the overall mortality for the study was somewhat lower than for previous large studies of thrombolytic agents in AMI, this is of particular concern. The chief inclusion criterion for the study was the presence of electrocardiographic evidence of AMI. A query of the database found 586 subjects (3.5% of all subjects in the ITT group) in whom electrocardiographic confirmation of AMI was absent or missing, or in whom the diagnosis was confirmed by cardiac enzymes alone. By a ratio of 1.2 to 1, there were more of these subjects in the TNK treatment group relative to the t-PA group. Overall, this is a relatively small fraction of subjects that would not be expected to importantly affect the study results.

Treatment Assignment and Compliance

Table 27 summarizes compliance with study drug administration for the ITT population. For the study overall, > 96% of subjects received a TNK bolus, t-PA bolus and t-PA infusion.

Dose Administered Versus Planned Dose

The planned dose is defined as the dose determined to be given on the basis of estimated weight. Potential causes of disparities between planned and administered doses include pharmacy and nursing errors, rapid changes in clinical course and/or unanticipated decisions in patient management. There was minimal disparity between the planned doses of study agents and the doses actually administered. Specifically, 93.5% of subjects in the TNK group received 95%–105% of the their planned dose. Similarly, 94.7% of subjects in t-PA group received 95%–105% of their planned dose. Overdose relative to the planned bolus dose of TNK (or the corresponding placebo in subjects assigned to t-PA) occurred in 1.6% of subjects in the both the TNK and t-PA groups. Overdose of the planned dose of t-PA (in subjects assigned to t-PA or corresponding placebo in the TNK arm) was <1% for both groups. Under-dosing (< 95% of planned dose) was observed in roughly 2% of subjects in both groups.

Dose Administered Versus "Ideal" Dose

The planned dose is determined by the medical staff. It is based on the best estimate of weight: actual weight (if obtained), historical weight, or bedside-estimated weight. The "ideal" dose is calculated on the basis of the actual weight, recorded in-hospital, when available. Only 53% of subjects had a recorded weight.

Thus, potential reasons for discrepancies between "ideal" dose and administered dose include all of the clinical factors noted above (i.e., pharmacy/nursing errors, changes in clinical status/management), as well as over- and under-estimates of weight. In a stepped dosing scheme, these errors affect the ideal dose when they cause migration from one dosing tier to another.

Table 27: Study Agent Administration – Relation Between Doses Planned, Doses Received, and "Ideal" Dose, ASSENT II

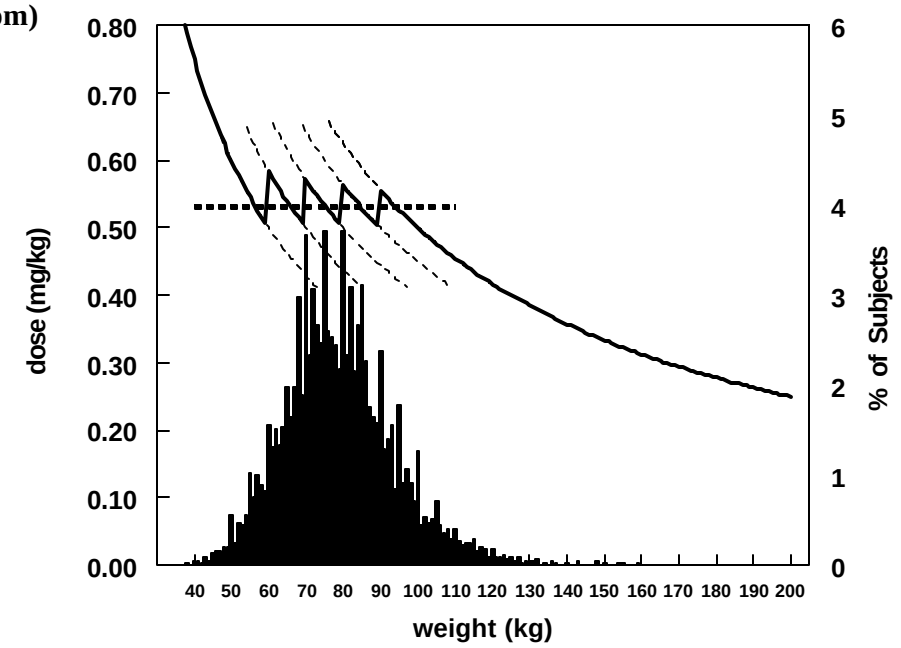
All subjects (actual weight recorded or unrecorded)	**n = 8461**	**n = 8488**
Received <u>any</u> agent, n (%)		
Subject received TNK bolus (%)	8199 (96.9)	
Subject received TNK dummy bolus (%)		8259 (97.3)
Subject received t-PA bolus (%)		8252 (97.2)
Subject received t-PA dummy bolus (%)	8201 (96.9)	
Subject received t-PA infusion (%)		8232 (97.0)
Subject received t-PA dummy infusion (%)	8172 (96.6)	
Percentage of planned dose TNK or corresponding dummy, n (%)		
< 95%	171 (2.0)	167 (2.0)
95-105%	7908 (93.5)	7956 (93.7)
> 105%	132 (1.6)	138 (1.6)
dose not given /not planned	250 (3.0)	227 (2.7)
Percentage of planned dose of t-PA or corresponding dummy, n (%)		
< 95%	155 (1.8)	154 (1.8)
95-105%	7992 (94.5)	8036 (94.7)
> 105%	45 (0.5)	56 (0.7)
dose not given /not planned	269 (3.2)	242 (2.8)
Subjects with actual weight recorded, n (%)	4487 (53.0)	4513 (53.2)
Percentage of ideal dose TNK or corresponding dummy, n (%)		
< 95%	339 (7.6)	363 (8.0)
95-105%	3522 (78.5)	3532 (78.3)
> 105%	520 (11.6)	535 (11.9)
dose not given /not planned	106 (2.4)	83 (1.8)
Percentage of ideal dose of t-PA or corresponding dummy, n (%)		
< 95%	118 (2.6)	141 (3.1)
95-105%	4095 (91.3)	4111 (91.1)
> 105%	153 (3.4)	164 (3.6)
dose not given /not planned	121 (2.7)	97 (2.1)

CBER performed an analysis of dose administered versus ideal dose for subjects with actual weights recorded (Table 27, bottom). (The sponsor performed a similar analysis, but used estimated weight in place of actual weights for subjects without recorded weights.) With respect to t-PA dosing, 91% of subjects in both groups received a t-PA dose (or corresponding placebo dose) within 5% of the ideal dose. Approximately 6-7% were divided between doses that were < 95% or >105% of the ideal dose. In contrast, only 78% of subjects received 95%–105% of their ideal TNK dose. Roughly 8% of subjects received an insufficient dose of TNK (or placebo if assigned to t-PA), and ~12% were overdosed. Thus, for the t-PA group, the ratio of subjects incorrectly dosed versus those correctly dosed was ~ 1:13. For the TNK group, the corresponding ratio was ~1:4, with excessive doses outnumbering insufficient doses by a ratio of 3:2. The sponsor explained the propensity towards TNK overdose on the basis of possible

weight loss of subjects subsequent to hospital admission, and the greater chance of error associated with the five-step TNK dosing approach.

Although weight loss could have been a factor leading to overdosage of TNK (or TNK placebo in subjects assigned to t-PA), CBER believes that the stepped dosing paradigm is the principal factor responsible for this observation. The design of the weight break points is such that rounding weights *down* to the nearest 10 kg has *no effect* with respect to TNK dose, whereas rounding *up* to the nearest 10 kg (in subjects who weigh <90 kg) *always* increases the dose. Moreover, the smallest possible error in the direction of overdose is 11.1% (which would result from administering 50 mg TNK when the ideal dose is 45 mg). Thus, *by design*, the rounding of weights to the nearest 10 kg results in overdosage half the time, but is unlikely to lead to under-dosing.

Figure 8: Effect of Stepped Dosing Paradigm on Weight-Adjusted Dose (Top) Weight Distribution for 9028 Subjects With Recorded Weights in ASSENT II (Bottom)



This problem is compounded by the tendency for observers to round patients' weights to the nearest 10 kg. For the present study, this is illustrated in Figure 8. The bottom graph shows the weight distribution, in 1 kg increments, for the 9028 subjects with recorded weights as percent of subjects (right Y-axis). Major peaks at 10 kg weights and minor peaks at 5 kg weights are apparent. For the stepped TNK dose scheme, the "ideal" dose is shown as a function of weight by the saw-tooth line at the top of the figure (left Y-axis). The horizontal dotted line at 0.53 mg/kg represents the target proportionality between dose and weight. Weight-adjusted doses for patients whose weights are overestimated fall on the curved dotted lines extending above the solid saw-tooth line; patients with underestimated weights receive weight-adjusted doses that fall on the curved dotted lines below the solid saw-tooth line. Subjects with weights evenly divisible by 10 kg also fall on the cusps of the dosing paradigm. These subjects will receive special consideration in CBER's analyses of efficacy and safety.

Integrity of the Blind

The treatment assignments of 50 and 31 subjects in the TNK and t-PA groups, respectively, were unblinded by investigators. An additional 34 subjects from the TNK group and 46 subjects from the t-PA group were unblinded to the Leuven Safety Group but did not fulfill the Category 1 criteria (serious, unexpected, study drug–related adverse events); therefore, the investigators were not unblinded for these events.

Reviewer's Comment(s): The extent of unblinding was minimal. Moreover, the impact of unblinding on the primary 30-day endpoint of mortality is unlikely to be consequential.

Study Population: Baseline Characteristics

Table 28 summarizes enrollment by country for the ITT population in descending order of numbers of subjects. The U.S. accounted for 21.6% of the total ITT population.

Table 28: Enrollment by Country (ITT Population), ASSENT II

Country	N	%	Country	N	%	Country	N	%
US	3660	21.6%	UK	451	2.7%	South Africa	230	1.4%
Israel	1557	9.2%	Poland	436	2.6%	United Arab Emirates	218	1.3%
Canada	1106	6.5%	Greece	380	2.2%	Finland	139	0.8%
Italy	1075	6.3%	France	355	2.1%	Mexico	137	0.8%
Sweden	1070	6.3%	Argentina	303	1.8%	Switzerland	121	0.7%
Australia	871	5.1%	Denmark	288	1.7%	Ireland	121	0.7%
Netherlands	852	5.0%	Brazil	280	1.7%	Austria	118	0.7%
Germany	771	4.5%	Portugal	261	1.5%	Turkey	103	0.6%
Belgium	770	4.5%	New Zealand	249	1.5%	Czech Republic	39	0.2%
Spain	752	4.4%	Norway	236	1.4%			
						TOTAL	**16949**	**100.0%**

Baseline characteristics for the ITT population are summarized in Table 29. For the study as a whole, median age was 61 years, with 25th and 75th quartiles of 52 and 70 years, respectively. Females comprised 23% of the ITT population, with a median age ~10 years older than their male counterparts. Race was reported as Caucasian in the vast majority of subjects (92-93%), with approximately 1.4% reporting African ancestry, 0.98% reporting Asian origin, and 5.07% reporting "other." Case Report Forms were not designed to capture Hispanic origin, and the proportions of Hispanic subjects are unknown.

Certain demographic characteristics and baseline disease status factors are associated with a poor prognosis in AMI. These include advanced age, female gender, lower systolic blood pressure, elevated heart rate, diabetes and prior MI. Anterior MI location is associated with a worse prognosis, independent of infarct size.

As expected for a study of this size, baseline characteristics were generally very well balanced. There were slight imbalances in important baseline characteristics that favor one treatment group or the other, and these are summarized below:

Favoring prognosis in the t-PA group:

- slightly lower prevalence of diabetes (15.7% vs. 16.4%, t-PA vs. TNK, respectively)

Favoring prognosis in the TNK group:

- proportion of females slightly less (22.9% vs. 23.3%, TNK vs. t-PA, respectively)
- prevalence of previous MI slightly less (15.8% vs. 16.2%, TNK vs. t-PA, respectively)
- lower proportion of anterior MI location (39.5% vs. 40.3%, TNK vs. t-PA, respectively)

Favoring neither group:

- Age (60.99 vs. 61.03 years, TNK vs. t-PA, respectively – the mean age difference is 17 days)
- Baseline Killip Class I was recorded for 88.0% of subjects in the TNK group and 88.1% of subjects in the t-PA group. Baseline Killip Class IV was recorded for 0.41% of subjects in the TNK group and 0.42% of subjects in the t-PA group (no prognostic advantage to either group).
- Median times to treatment (MI symptom onset to study agent initiation) were 2.71 and 2.75 hours in the TNK and t-PA groups, respectively (mean difference = 1.4 minutes)
- Heart rate and blood pressure were essentially equivalent in both groups

Reviewer's Comment(s): The imbalances noted are quite small, and represent very small numbers of subjects. Overall, they would not be expected to materially affect the study results.

Concomitant and excluded medications

Concomitant medication use was balanced between study treatment groups. Within both groups, virtually all subjects received heparin (>99%) and the vast majority received aspirin, per protocol (>97%). β-

Table 29: Baseline Characteristics (ITT Population)

	TNK	t-PA
N	8461	8488
Characteristic		
age [years; median Q25-Q75]		
all	61 (52 - 70)	61 (52 - 70)
male	59 (50 - 68)	59 (51 - 68)
female	69 (60 - 75)	68 (59 - 75)
age > 75 [%]	12.4	12.6
male	8.8	9.0
female	24.5	24.5
number (%) male	77.1	76.7
race [n (%)]		
Caucasian	92.6	92.5
African descent	1.4	1.4
Asian	0.9	1.0
other	5.0	5.1
subject mass [kg; median Q25-Q75]	78 (69-87)	78 (69-87)
subject mass [%]		
< 60 kg	7.5	7.7
60-69 kg	17.8	17.5
70-79 kg	28.8	28.8
80-89 kg	24.4	24.0
>=90 kg	21.5	22.0
infarct location [%]		
anterior	39.5%	40.3%
inferior	55.5%	54.9%
lateral	3.1%	3.1%
posterior	0.5%	0.7%
LBBB	0.9%	0.5%
missing	0.5%	0.5%
HR [bpm, median Q25-Q75]	72 (62-85)	73 (62-85)
systolic BP [mmHg, median Q25-Q75]	133 (120-150)	133 (119-150)
Killip Class [n (%)]		
I	7428 (88.0)	7465 (88.1)
II	887 (10.5)	874 (10.3)
III	93 (1.1)	98 (1.2)
IV	35 (0.4)	36 (0.4)
previous medical history [%]		
HTN	37.7	38.6
diabetes	16.4	15.7
previous MI	15.8	16.2
prior CABG	3.9	3.9
prior PTCA	5.1	5.6
current smoker	44.8	44.2
ex-smoker	27.7	27.6
time to treatment [hours, median Q25-Q75]	2.71 (1.87-3.83)	2.75 (1.85-3.92)
time to treatment [%]		
0 - 2 hours	30.0	30.4
> 2 - 4 hours	48.1	46.3
> 4 - 6 hours	19.1	20.5
> 6 hours	2.8	2.8

blocker and nitrate use was common, reported in 81% and 73% of subjects overall, respectively. Use of abciximab and other marketed glycoprotein IIb/IIIa antagonists within 24 hours after randomization was discouraged. Overall, 7.6% of subjects in both groups received these medications during their hospitalization. Approximately one-fifth of subjects received the antiplatelet agents ticlopidine/clopidogrel, and a similar proportion received low molecular weight heparins.

Table 30: Concomitant Medication Use, ASSENT II

	TNK	t-PA
Aspirin		
£12 hours (%)	44.6%	45.1%
Upon randomization (%)	58.8%	59.2%
*** at either time**	97.3%	97.5%
Heparin		
Bolus (%)	94.5%	94.4%
Infusion (%)	99.3%	99.2%
GP IIb/IIIa antagonists (%)	7.6%	7.6%
Ticlopidine/clopidogrel (%)	22.5%	22.4%
*** Re-administration of thrombolytics (%)**	2.7%	2.3%
*** Other thrombolytics (%)**	1.5%	1.2%
Low molecular weight heparin (%)	21.1%	21.7%
IV nitrates (%)	72.8%	72.2%
Beta blockers (%)	80.7%	80.8%
ACE inhibitors (%)	53.2%	53.8%
Angiotensin II inhibitors (%)	1.8%	1.9%
Statins (%)	31.4%	31.8%

* CBER analysis

Reviewer's Comment(s):
CBER analyzed the subset of subjects who received GP IIb/IIIa inhibitors (7.6% of subjects, overall, Table 31). Over 99% of these subjects underwent invasive cardiovascular procedures (coronary cineangiography, PTCA, stent, CABG or intra-aortic balloon pump): ~15% underwent PTCA alone and ~80% underwent stent deployment. The co-administration of GP IIb/IIIa inhibitors and thrombolytic agents would be expected to be associated with an increased risk of bleeding. In this study, however, administration of the IIb/IIIa agent generally occurred in association with invasive procedures performed several days after AMI, and therefore too long after administration of the thrombolytic agent to importantly interact and increase bleeding. Conversely, CBER found that administration of a GP IIb/IIIa inhibitor was recorded on Day 0 in ~22% of the subjects who received these agents, or in ~ 1.7% of subjects overall. This subgroup will be analyzed separately for efficacy and safety (see below).

Table 31: GP IIb/IIIa Use, ASSENT II

	TNK	t-PA
GP IIb/IIIa Use (N)	639	640
Any procedure (N)	634	637
%	99.2%	99.5%
PTCA or stent	608	604
	95.1%	94.4%
PTCA without stent (N)	113	81
%	17.7%	12.7%
Stent (N)	495	523
%	77.5%	81.7%
GP IIb/IIIa agent administration recorded on Day 0 (N)	152	137
%	23.8%	21.4%

Efficacy Results

Primary Efficacy Results

The primary efficacy endpoint was 30-day mortality, to be analyzed using a non-parametric covariate-adjusted method. The five predefined covariates (all known to have prognostic significance in AMI) included age, infarct location, Killip class, systolic blood pressure and heart rate. Mortality rates were also analyzed using an unadjusted method and in an exploratory analysis using logistic regression (using the same five covariates as above).

The ITT population included 8461 and 8488 subjects in the TNK and t-PA groups, respectively. Thirty-day vital status was available for all except 3 subjects in each group lost to follow-up. There were 1045 deaths observed in the ITT population: 521 in the TNK group and 524 in the t-PA group.

Using the non-parametric covariate-adjusted method, the relative risk of 30-day mortality for TNK over accelerated t-PA is 1.004. The upper limit of the one-sided 95% CI is 1.104, within the pre-specified upper limit of 1.14. The unadjusted relative risk was 0.9974, slightly better with respect to TNK. The one-sided upper 95% confidence limit of 1.101 – also within the CBER-proposed acceptable limit of 1.14. Logistic regression results were similar. CBER was able to verify the results of the sponsor's statistical analyses.

Table 32: Thirty-Day Mortality, ASSENT II (Relative Risk; Intent to Treat Population)

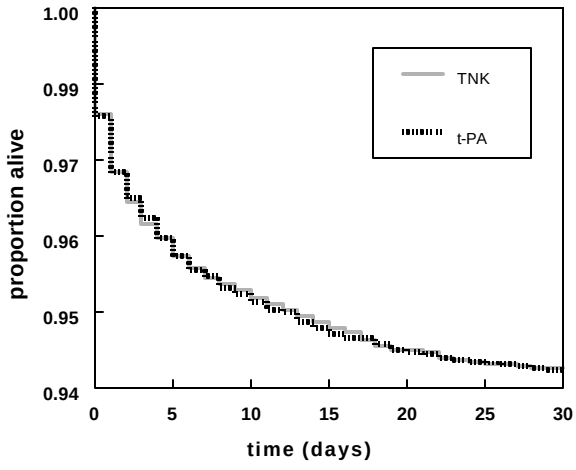
	TNK			t-PA			RR	90% CI
	n	deaths	%	n	deaths	%		
non-parametric adjusted rate								
	8458	521	6.18%	8485	524	6.15%	1.004	(0.914, 1.104)
unadjusted rate								
	8458	521	6.16%	8485	524	6.18%	0.997	(0.904, 1.101)

With respect to absolute mortality, the sponsor's non-parametric covariate-adjusted analysis shows an absolute difference in 30-day mortality of 0.028% (TNK > t-PA). The upper limit of the one-sided 95% CI is 0.609%. These results are similar to those obtained using the unadjusted method and the logistic regression analysis. Thus, all three results are within 1% for the upper limit of the 95% CI.

Time-to-Event Analysis

The Kaplan-Meier time-to-event analysis for the ITT population shows virtual superimposition of the survival curves for the two treatment groups, indicating no difference in time to death between the groups.

Figure 9: Kaplan-Meier Analysis – Survival (ITT Population), ASSENT II



Exploratory Analysis on the Primary Endpoint – Subjects Lost to Follow-Up

Only a trivial number of subjects were lost to follow-up (3 per treatment arm). CBER performed a "worst-case" analysis, based on assumptions that the 3 subjects in the TNK group lost to follow-up had died by Day 30, whereas the 3 subjects in the t-PA group with missing follow-up had survived. For this analysis, the unadjusted relative risk is 1.003. The upper limit of the one-sided 95% CI is 1.128, remaining within the pre-specified upper boundary of 1.14.

Exploratory Analysis on the Primary Endpoint – First versus Second Half of Enrollment

CBER assessed mortality rates in the with respect to time of enrollment: first and second half (Table 33). The median date of study entry was June 17, 1998. In the first half of the study, mortality tended to be lower in the TNK group (RR = 0.882 in favor of TNK). In the second study half, however, the trend was reversed. Mortality tended to be higher in the TNK group, with RR = 1.132. The difference in point estimates between study halves is so extreme that the 95% confidence intervals of each study half *exclude* the point estimate of the other half.

Table 33: 30-Day Mortality by Time of Enrollment

	TNK			t-PA			RR	95% CI
	n	deaths	%	n	deaths	%		
*** study half**								
first	4228	249	5.89%	4208	281	6.68%	0.882	(0.748, 1.04)
second	4230	272	6.43%	4277	243	5.68%	1.132	(0.957, 1.338)

*** CBER Analyses**

CBER examined major baseline demographic and disease characteristics of the first and second study halves for both treatment groups, to identify potential imbalances that may have accounted for inconsistent efficacy between study halves. In the t-PA group, there was imbalance between the first and second study halves with respect to the numbers of subjects > 75 years of age (13.3% and 11.9%, respectively). The imbalance was directionally in favor of higher mortality in the first study half, as observed. However, further analyses showed that the key difference in mortality between study halves in the t-PA group was in subjects *younger* than 75 years of age. In this group comprising ~88% of the t-PA study population, the mortality rates were 4.9% and 3.7% for the first and second study halves, respectively. Thus, age imbalance does not appear to account for the disparity/reversal in mortality rates between study halves, and the reason(s) for this observation remain unknown.

Subgroup Analyses

Thirty-day mortality subgroup analyses are shown in Table 34, Table 35, Table 36, Table 37 and Table 38. In the interest of providing a logical presentation of the results, CBER's exploratory analyses are intermixed with the sponsor's analyses in some tables. CBER verified the results of the sponsor's analyses, and CBER's analyses are denoted by asterisks.

Age, Gender and Race

Thirty-day mortality was similar between the two treatment groups across age, gender and race subgroups (Table 34). Consistent with results of previous large trials of thrombolytic agents in AMI, advanced age and female gender were associated with worse outcome. For these high-risk subgroups, mortality rates were similar in the TNK and t-PA treatment groups.

Table 34: 30-Day Mortality – Age, Gender and Race Subgroups

	TNK			t-PA			RR	95% CI
	n	deaths	%	n	deaths	%		
Overall	8458	521	6.16%	8485	524	6.18%	0.997	(0.887, 1.122)
Age (years)								
age <=75	7408	338	4.56%	7410	318	4.29%	1.063	(0.915, 1.235)
age >75	1047	182	17.38%	1070	206	19.25%	0.903	(0.754, 1.081)
*** Age (years)**								
< 53	2282	42	1.84%	2251	39	1.73%	1.062	(0.69, 1.636)
53 - 61	1962	55	2.80%	2025	61	3.01%	0.931	(0.65, 1.333)
62 - 70	2173	137	6.30%	2091	117	5.60%	1.127	(0.887, 1.431)
> 70	2041	287	14.06%	2118	307	14.49%	0.970	(0.836, 1.126)
Gender								
male	6518	327	5.02%	6505	314	4.83%	1.039	(0.894, 1.209)
female	1940	194	10.00%	1980	210	10.61%	0.943	(0.784, 1.134)
*** Gender-Age**								
male; age <= 75	5945	234	3.94%	5915	220	3.72%	1.058	(0.883, 1.268)
male; age > 75	571	92	16.11%	585	94	16.07%	1.003	(0.771, 1.305)
female; age <= 75	1463	104	7.11%	1495	98	6.56%	1.084	(0.831, 1.415)
female; age > 75	476	90	18.91%	485	112	23.09%	0.819	(0.64, 1.048)
Race								
Caucasian	7636	468	6.13%	7637	472	6.18%	0.992	(0.876, 1.122)
African descent	116	5	4.31%	114	3	2.63%	1.638	(0.401, 6.694)
Asian	76	6	7.89%	86	9	10.47%	0.754	(0.281, 2.022)
other	415	35	8.43%	421	26	6.18%	1.366	(0.837, 2.227)

*** CBER Analyses**

Reviewer's Comment(s): CBER also analyzed a combined gender/age variable (i.e., two dichotomous variables: gender male/female; age over/under 75 years), and no important differences in mortality between treatment groups were identified.

Weight and Body Mass Index (BMI)

Registry data and results of large clinical trials have shown that low-weight is associated with higher morbidity and mortality after AMI. This is due in part to overrepresentation of females and older subjects in lower weight groups; however, lower weight patients have worse outcomes even after adjusting for these factors. Low weight patients have an increased incidence of bleeding, tend to receive treatment later, and are less likely to undergo coronary arteriography, percutaneous coronary interventions and coronary artery bypass surgery.

Consistent with observations in prior studies, mortality tended to be higher in the lower body weight subgroups of each treatment group (Table 35). Within all weight classes, however, mortality rates were similar between the TNK and t-PA treatment groups.

Reviewer's Comment(s): Because of the interaction between weight and gender, CBER performed additional analyses to assess morality rates for TNK and t-PA within gender-specific body mass index (BMI) tertiles. This was done in part to determine whether the stepped dosing paradigm for TNK resulted

Table 35: 30-Day Mortality – Weight and Body Mass Index Subgroups

	TNK			t-PA			RR	95% CI
	n	deaths	%	n	deaths	%		
Weight								
< 67	1556	157	10.09%	1575	149	9.46%	1.067	(0.862, 1.32)
>= 67	6874	357	5.19%	6879	370	5.38%	0.966	(0.838, 1.112)
Weight								
< 60	629	75	11.92%	653	67	10.26%	1.162	(0.852, 1.586)
60 - 69	1500	117	7.80%	1479	132	8.92%	0.874	(0.689, 1.109)
70 - 79	2427	136	5.60%	2437	134	5.50%	1.019	(0.808, 1.285)
80 - 89	2061	97	4.71%	2026	110	5.43%	0.867	(0.664, 1.131)
>= 90	1813	89	4.91%	1859	76	4.09%	1.201	(0.89, 1.62)
*** Body Mass Index (BMI) tertile**								
thin	2546	151	5.93%	2540	151	5.94%	0.998	(0.802, 1.242)
medium	2543	131	5.15%	2559	134	5.24%	0.984	(0.778, 1.244)
heavy	2570	112	4.36%	2586	124	4.80%	0.909	(0.708, 1.167)
*** Gender and BMI**								
male - thin	1995	94	4.71%	1939	90	4.64%	1.015	(0.766, 1.346)
male - medium	1959	86	4.39%	1989	90	4.52%	0.970	(0.727, 1.295)
male - heavy	2187	116	5.30%	2251	131	5.82%	0.911	(0.715, 1.162)
female - thin	551	57	10.34%	601	61	10.15%	1.019	(0.724, 1.435)
female - medium	584	45	7.71%	570	44	7.72%	0.998	(0.67, 1.488)
female - heavy	594	44	7.41%	572	49	8.57%	0.865	(0.585, 1.278)

*** CBER Analyses**

in decreased efficacy for patient subpopulations at the extremes of body type. No sub-groups were identified with important disparities in mortality rates between the TNK and t-PA groups.

Geographical Location and Size of Sites

Local medical practices may influence outcomes in AMI. Such practices may be specific to a particular country, locale, center or investigator. The sponsor examined mortality rates for US versus non-US sites and European versus non-European sites, and no important patterns emerged (Table 36).

Reviewer's Comment(s): CBER performed an analysis of mortality by site size as an attempt to differentiate sites by the overall volume of AMI patients treated at hospitals/locations. CBER divided the sites into tertiles on the basis of numbers of subjects enrolled, and found no differences in mortality between TNK and t-PA groups (Table 36). CBER also analyzed mortality by country. Table 36 shows mortality results for the 18 countries contributing the largest numbers of subjects. Only in Australia was there a significant difference in mortality rates between TNK and t-PA. Given the multiplicity of analyses, and the fact that mortality in the Australia TNK group was in line with other countries (a strikingly low mortality rate in the Australian t-PA-treated subjects appeared to account for the difference), this is not cause for concern.

Table 36: 30-Day Mortality – Geographic Location and Site Size

	TNK			t-PA			RR	95% CI
	n	deaths	%	n	deaths	%		
United States								
No	6647	412	6.20%	6638	424	6.39%	0.970	(0.851, 1.106)
Yes	1811	109	6.02%	1847	100	5.41%	1.112	(0.854, 1.447)
Europe								
No	4338	262	6.04%	4373	246	5.63%	1.074	(0.907, 1.271)
Yes	4120	259	6.29%	4112	278	6.76%	0.930	(0.789, 1.095)
*** Site size tertile**								
1 - 18	2737	188	6.87%	2756	176	6.39%	1.076	(0.882, 1.312)
18 - 36	2872	171	5.95%	2890	186	6.44%	0.925	(0.757, 1.131)
> 36	2849	162	5.69%	2839	162	5.71%	0.996	(0.807, 1.231)
*** Country**								
US	1811	109	6.02%	1847	100	5.41%	1.111	(0.854, 1.447)
Israel	781	34	4.35%	776	30	3.87%	1.126	(0.696, 1.821)
Canada	549	31	5.65%	557	34	6.10%	0.925	(0.577, 1.483)
Italy	540	25	4.63%	535	28	5.23%	0.885	(0.523, 1.497)
Sweden	528	30	5.68%	542	40	7.38%	0.770	(0.487, 1.217)
Australia	436	28	6.42%	435	11	2.53%	**2.540**	**(1.281, 5.036)**
Netherlands	432	27	6.25%	419	21	5.01%	1.247	(0.716, 2.171)
Belgium	392	27	6.89%	378	35	9.26%	0.744	(0.459, 1.204)
Germany	381	26	6.82%	390	33	8.46%	0.806	(0.492, 1.322)
Spain	374	19	5.08%	378	28	7.41%	0.686	(0.39, 1.206)
UK	232	27	11.64%	219	15	6.85%	1.699	(0.929, 3.107)
Poland	217	11	5.07%	219	16	7.31%	0.693	(0.33, 1.461)
Greece	188	9	4.79%	191	12	6.28%	0.762	(0.329, 1.766)
France	177	5	2.82%	178	10	5.62%	0.503	(0.175, 1.441)
Argentina	149	12	8.05%	154	21	13.64%	0.591	(0.301, 1.157)
Denmark	144	11	7.64%	144	13	9.03%	0.846	(0.392, 1.826)
Brazil	137	12	8.76%	142	16	11.27%	0.777	(0.382, 1.582)
Portugal	130	9	6.92%	131	6	4.58%	1.512	(0.554, 4.126)

*** CBER Analyses**

Heart Rate, Blood Pressure, MI Location, Killip Class and Time to Treatment

Tachycardia, hypotension, anterior MI location, advanced Killip class and delayed time to treatment are associated with worse outcome in AMI, and these associations are apparent in the ASSENT II data (Table 37). Mortality rates are similar between TNK and t-PA groups across these subgroups, however, with heart rate and time to treatment the apparent exceptions (see below).

Heart Rate – At heart rates of 70 and below, mortality tended to be somewhat higher in the TNK group compared to the t-PA group.

Reviewer's Comment(s): CBER evaluated these data more closely, and found that this trend alternated across successive 10 bpm heart rate categories, suggesting a lack of a pathophysiologic or pharmacologic mechanism underlying this observation. Random variation provides the most likely the explanation for this finding.

Time to Treatment – As observed in previous large studies of thrombolytic agents in AMI, mortality increased with increasing "symptom-to-needle" times; however, there was a difference in mortality (favoring TNK) for subjects treated > 4 hours after symptom onset (RR = 0.766, p = 0.018 – not corrected for multiplicity of analyses). This association did not persist, however, in the sponsor's multivariate analyses that included other independent predictors of mortality.

Table 37: 30-Day Mortality – Heart Rate, Blood Pressure, MI Location, Killip Class and Time to Treatment Subgroups

	TNK			t-PA			RR	95% CI
	n	deaths	%	n	deaths	%		
Heart rate (bpm)								
<60	1454	72	4.95%	1456	56	3.85%	1.287	(0.915, 1.812)
60 - 70	1971	86	4.36%	1911	67	3.51%	1.245	(0.91, 1.702)
70 - 80	1900	89	4.68%	1942	118	6.08%	0.771	(0.59, 1.008)
80 - 90	1531	102	6.66%	1500	92	6.13%	1.086	(0.827, 1.427)
90 - 100	815	60	7.36%	837	73	8.72%	0.844	(0.608, 1.171)
> 100	774	110	14.21%	820	115	14.02%	1.013	(0.795, 1.291)
*** Systolic blood pressure (mmHg)**								
< 90	192	57	29.69%	179	47	26.26%	1.131	(0.814, 1.57)
90 - 99	270	32	11.85%	290	36	12.41%	0.955	(0.611, 1.492)
100 - 139	4374	256	5.85%	4321	260	6.02%	0.973	(0.823, 1.15)
140 - 174	3361	162	4.82%	3390	163	4.81%	1.002	(0.811, 1.239)
> 174	251	11	4.38%	293	15	5.12%	0.856	(0.401, 1.83)
MI location								
anterior	3332	266	7.98%	3408	279	8.19%	0.975	(0.83, 1.146)
other	5112	254	4.97%	5059	245	4.84%	1.026	(0.865, 1.218)
Killip Class								
I	7425	351	4.73%	7462	359	4.81%	0.983	(0.851, 1.134)
II	887	120	13.53%	874	117	13.39%	1.011	(0.797, 1.281)
III	93	27	29.03%	98	24	24.49%	1.185	(0.74, 1.899)
IV	35	18	51.43%	36	22	61.11%	0.842	(0.556, 1.273)
Time to treatment (hours)								
< 2	2519	125	4.96%	2563	125	4.88%	1.017	(0.799, 1.296)
> 2 - 4	4036	256	6.34%	3902	214	5.48%	1.157	(0.97, 1.379)
> 4	1832	129	7.04%	1970	181	9.19%	0.766	(0.617, 0.952)
*** Time to treatment (hours)**								
> 4 - 6	1597	119	7.45%	1730	158	9.13%	0.816	(0.65, 1.025)
> 6	235	10	4.26%	240	23	9.58%	0.444	(0.216, 0.913)

*** CBER Analyses**

Reviewer's Comment(s): CBER performed additional analyses on time to treatment. CBER subdivided subjects treated > 4 hours after symptom onset into those treated > 4 to ≤ 6 hours and those treated > 6 hours after symptom onset (the latter subgroup violated protocol inclusion criteria).

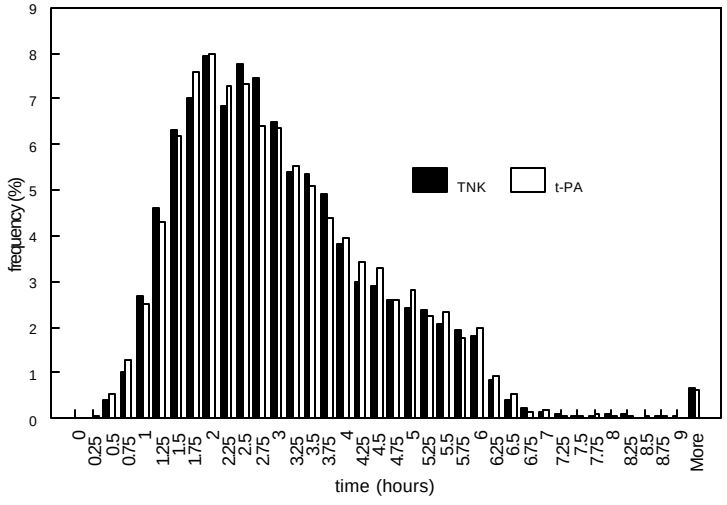
- Treatment > 6 Hours After Symptom Onset

Within the TNK treatment group, there were 235 subjects treated after 6 hours (2.8% of the total ITT population), and the mortality in this subgroup was only 4.26%. This mortality rate is strikingly low, and

represents the lowest mortality rate in any "symptom-to-needle" stratum. These results are not consistent with the general trend of increasing morality with increasing symptom-to-needle time within the group of TNK-treated subjects, and they are counterintuitive, because survival after MI is generally inversely related to time-to-treatment. Thus, CBER considered other factors that may have contributed to the lower than expected mortality rate in this subgroup.

Mortality would be lower than expected if a substantial proportion of subjects treated > 6 hours after symptom onset were falsely diagnosed with AMI. Misdiagnosis may be more likely in subjects with delayed presentation, because evidence of AMI is time-sensitive. For the 475 subjects treated > 6 hours after symptom onset, AMI was confirmed by both ECG and cardiac enzymes in 84.4% of subjects. For subjects treated within 6 hours of symptom onset, 91.0% of subjects had confirmation of AMI by both ECG and enzymes. Thus, although proper documentation of AMI was less frequent in subjects presenting > 6 hours after symptom onset, the magnitude of the problem was not sufficient to explain the low mortality rate in this subgroup.

Figure 10: Frequency Distribution of Symptom-to-Needle Time (Hours)



Protocol-mandated limits in time to enrollment have the potential to induce investigators to record erroneous times of symptom onset, in order to increase enrollment (i.e., "rounding" the time of symptom onset to a slightly later time obtain a symptom-to-randomization time within the protocol-specified 6 hours). To consider this possibility, CBER assessed the symptom-to-needle time frequency distribution for both treatment groups (Figure 10). As expected, there is an apparent "drop-off" between 6.0 and 6.25 hours; however, there is no persuasive evidence of a peak at 6 hours. This suggests that significant numbers of subjects who presented at somewhat > 6 hours were not erroneously classified as treated within 6 hours. It is likely that the peak at time > 9 hours (far right) represents subjects who had a prolonged and/or stuttering symptom course, subjects with an erroneously recorded time of symptom onset, and subjects who truly had a symptom-to-needle time > 9 hours. The latter subgroup represent a selected AMI population, in that the ability to survive > 9 hours after symptom onset and be enrolled in a clinical trial would be expected to portend a more favorable prognosis.

The case report forms did not capture subjects with a stuttering symptom course; therefore, this potentiality can not be further assessed. Errors in recording time(s) of symptom onset and/or bolus administration may be difficult to detect, but there are occasional examples where commission of an error appears to be likely (i.e., misinterpretation of midnight [0:00 versus 24:00 hours], possible misreading of handwriting). These were found in only a tiny minority of subjects, however, and are unlikely to account for the very low mortality rate in this subgroup. The potential for selection bias, although real, can not be

addressed by the data. In light of these unknowns and potential limitations, and given that these subjects may have violated protocol inclusion criteria, they are not considered further.

- Treatment 4 – 6 Hours After Symptom Onset

CBER also performed a multivariate analysis for the 4–6 hour time to treatment subgroup, using the same cofactors utilized for analysis of the primary mortality endpoint (age, Killip class, heart rate, systolic blood pressure and infarct location). This group encompassed ~20% of the total study population. The mortality advantage for TNK was not statistically significant when the cofactors were included in the analyses. (There were important imbalances between treatment groups with respect to baseline heart rate and age, favoring prognosis in the TNK group.)

Coexistent Diseases; Smoking

Diabetes and history of prior MI are associated with a poor prognosis in AMI. Table 38 provides mortality rates for TNK and t-PA for the subgroups with a history of hypertension, diabetes, previous MI and previous CABG, as well as current smoking. For subjects with a history of prior CABG, mortality tended to be higher in the TNK group; however, these subjects represented a very minor fraction of the study population overall (3.9%).

Table 38: 30-Day Mortality – Coexistent Diseases; Current History of Smoking

	TNK			t-PA			RR	95% CI
	n	deaths	%	n	deaths	%		
Hypertension								
No	5263	264	5.02%	5199	271	5.21%	0.962	(0.816, 1.135)
Yes	3187	255	8.00%	3267	249	7.62%	1.050	(0.888, 1.241)
Diabetes								
No	7066	398	5.63%	7140	405	5.67%	0.993	(0.868, 1.136)
Yes	1383	121	8.75%	1329	116	8.73%	1.002	(0.786, 1.278)
Previous MI								
No	7116	389	5.47%	7098	402	5.66%	0.965	(0.843, 1.105)
Yes	1335	131	9.81%	1367	118	8.63%	1.137	(0.897, 1.441)
Previous CABG								
No	8123	488	6.01%	8136	495	6.08%	0.987	(0.875, 1.115)
Yes	327	32	9.79%	327	25	7.65%	1.280	(0.776, 2.111)
*** Smoker (current)**								
No	4622	363	7.85%	4686	364	7.77%	1.011	(0.879, 1.163)
Yes	3744	137	3.66%	3707	131	3.53%	1.035	(0.818, 1.31)

*** CBER Analyses**

Reviewer's Comment(s): In both treatment groups, smoking was associated with a 2-fold *reduction* in mortality. Although counter-intuitive, this association has been observed in previous larger studies of thrombolytic agents in AMI, and is likely due to differences in other baseline characteristics between smokers and non-smokers. Compared to subjects currently smoking, current non-smokers were more likely to be female (27.8% versus 17.1%, respectively), older (by ~10 years), and approximately twice as

likely to be diabetic. History of prior MI, CABG and PTCA are also more commonly associated with non-smokers (Table 39).

Table 39: Covariates Associated with Current Smoking (CBER Analysis)

	n	mortality (%)	female (%)	median age (years)	anterior MI location (%)	diabetic (%)	previous MI (%)	previous CABG (%)	previous PTCA (%)
Current smoker	7451	3.6%	17.1%	55	36.9%	10.6%	12.3%	1.9%	4.4%
Not a current smoker	9308	7.8%	27.8%	66	42.1%	20.3%	18.9%	5.4%	6.1%

Agents with the Potential to Exacerbate Bleeding; TNK/t-PA Excess

Concomitant use of antithrombotic agents, inhibitors of platelet aggregation and GPIIb/IIIa receptor blockers have the potential to lead to increased risk of bleeding. CBER analyzed mortality rates for the subgroups of subjects with reported use of glycoprotein IIb/IIIa antagonists, low molecular weight heparin (LMWH) and the anti-platelet agents ticlopidine/clopidogrel. Because of the short half-life of GPIIb/IIIa antagonists, the timing of GPIIb/IIIa administration has important ramifications with respect to potential interactions with thrombolytic agents. For example, administration of a GPIIb/IIIa agent on day 12 would not affect the risk of bleeding due to a thrombolytic agent administered on day 0. Although the day of GPIIb/IIIa administration was not recorded on the CRFs, CBER found that GPIIb/IIIa use was virtually always associated with percutaneous coronary interventions (PCI), the day of which *was* recorded. Thus, CBER defined an "early" GPIIb/IIIa use subgroup, based on GPIIb/IIIa use and the performance of PCI on Day 0 or Day 1. As shown in Table 40, subjects who received LMWH, ticlopidine/clopidogrel, and GPIIb/IIIa agents (the latter at *any* time) tended to have *lower* mortality than subjects who did not receive these agents. Importantly, no differences were apparent between the TNK and t-PA treatment groups in these subgroups. Mortality did not appear to be materially increased in the subgroup who received GPIIb/IIIa agents on Day 0 or Day 1. The numbers of subjects were too limited, however, to assess differences between treatment groups. CBER also analyzed mortality in the subset of subjects who received > 105% of their ideal TNK dose. These subjects are underreported, because the designation of a TNK "overdose" could be made only in the 53% of subjects who were actually weighed. Although this hypothesis can not be tested, it is possible that weight errors were more frequent for subjects in whom weight was never directly assessed, due to more compromised clinical status.

Table 40: 30-Day Mortality – Concomitant Use of Agents with the Potential to Exacerbate Bleeding; TNK/t-PA Excess

	TNK			t-PA			RR	95% CI
	n	deaths	%	n	deaths	%		
*** IIb/IIIa Antagonists**								
No	7801	489	6.27%	7832	497	6.35%	0.988	(0.875, 1.115)
Yes	639	28	4.38%	640	27	4.22%	1.039	(0.619, 1.742)
*** IIb/IIIa agent on Day 0**								
No	8305	511	6.15%	8343	516	6.18%	0.995	(0.884, 1.12)
Yes	153	10	6.54%	142	8	5.63%	1.160	(0.471, 2.857)
*** LMWH use**								
No	6659	472	7.09%	6632	463	6.98%	1.015	(0.897, 1.149)
Yes	1783	44	2.47%	1841	61	3.31%	0.745	(0.508, 1.091)
*** Ticlopidine/Clopidogrel**								
No	6514	489	7.51%	6552	495	7.55%	0.994	(0.881, 1.121)
Yes	1897	27	1.42%	1891	29	1.53%	0.928	(0.552, 1.562)
*** TNK >105% of planned dose (confirmed weights)**								
No	3860	149	3.86%	3894	127	3.26%	1.184	(0.938, 1.493)
Yes	519	6	1.16%	535	9	1.68%	0.687	(0.246, 1.917)
*** weight not confirmed**	4079	366	8.97%	4056	388	9.57%	0.938	(0.819, 1.075)

*** CBER Analyses**

Percutaneous Coronary Interventions

The use of PCI deserves special consideration in the analysis and interpretation of these study results. Primary PTCA, and more recently, PTCA accompanied by stent implantation, has been gaining popularity as a treatment strategy for AMI. In the present study, roughly 25% of subjects in both groups underwent PTCA, with or without stent implantation

Early PCI

A decision to enroll an AMI subject in this investigation was tantamount to a decision to manage a subject conservatively, i.e., a plan *not* to utilize primary PTCA or stenting as therapy for AMI. However, there were subjects in whom PCI was performed within a day of hospitalization. Presumably, some of these subjects underwent urgent primary PTCA and did not receive their assigned thrombolytic agent because of rapid clinical deterioration. Others may have undergone PCI soon after administration of the thrombolytic agent as a "bail out" procedure, for unremitting ischemia, pump failure, etc. Thus, excessive and early use of PCI could be interpreted as a sign of reduced efficacy, or an indicator that the most compromised subjects were selected out before treatment, and the study population is not representative of a true AMI population.

Delayed PCI

Percutaneous coronary interventions performed later during the course of hospitalization are used primarily to manage recurrent ischemia, or are performed on the basis of information obtained during hospitalization suggesting that PCI would prevent future symptoms and/or major coronary events.

PTCA is an integral part of stent implantation, and the CRFs were not designed in such a way as to compel investigators to make a distinction between PTCA without stent implantation, versus stent implantation with incidental PTCA. As such, there were numerous subjects in whom both PTCA and

stent implantation were reported. Specifically, ~75% of subjects who were included in the PTCA subgroup also belonged to the stent subgroup. Thus, CBER redefined the PTCA subgroup to exclude subjects who had undergone stent implantation, with a goal of identifying a "pure" PTCA subgroup. CBER also defined a new subgroup of subjects who underwent PTCA *or* stenting, because for both modalities, the intent is the same (utilization of a mechanical intervention to restore patency to a stenotic artery). The decision to use PTCA versus stent is largely dependent on physician preference, anatomical considerations, perceived risk of restenosis, etc.

Table 41: 30-Day Mortality in Subjects Undergoing Percutaneous Coronary Interventions

	TNK (n=8458)			t-PA (n=8485)			RR	95% CI
	n	deaths	%	n	deaths	%		
*** Stent**								
No	6831	483	7.07%	6803	491	7.22%	0.980	(0.868, 1.106)
Yes	1608	35	2.18%	1668	32	1.92%	1.135	(0.706, 1.823)
*** PTCA**								
No	7957	497	6.25%	8065	509	6.31%	0.990	(0.878, 1.115)
Yes	501	24	4.79%	420	15	3.57%	1.341	(0.713, 2.523)
*** Stent or PTCA**								
No	6347	462	7.28%	6396	477	7.46%	0.976	(0.863, 1.104)
Yes	2111	59	2.79%	2089	47	2.25%	1.242	(0.851, 1.814)

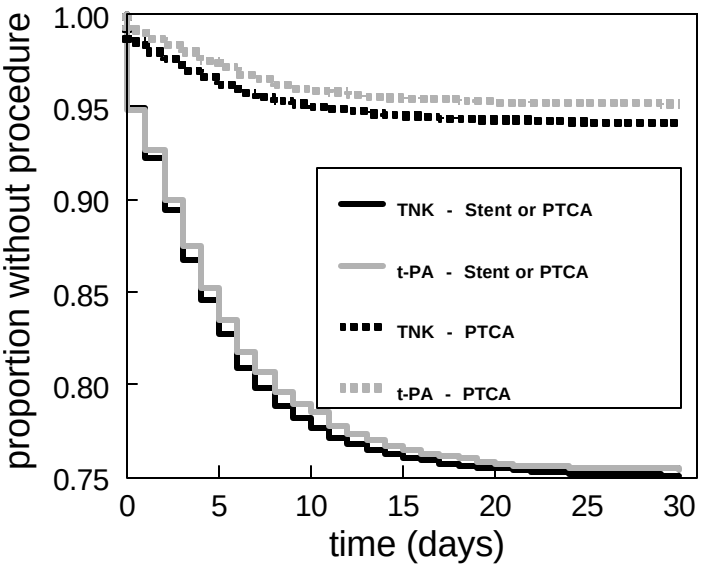
*** CBER Analyses**

Mortality was decreased for all PCI subgroups relative to subjects who did not have PCI (Table 41). There was a trend towards increased mortality in the PTCA subgroup of the TNK treatment group versus t-PA (RR = 1.341), although the numbers of subjects were quite limited. It is also apparent that PTCA (without stent implantation) was reported more commonly in the TNK group than the t-PA group (501 versus 420 subjects, 5.9% versus 4.9%, respectively). This was balanced by fewer stents reported in the TNK group, such that the combined PCI variable "stent or PTCA" occurred with roughly equal frequency in the TNK and t-PA groups. As noted above, the combined incidence is thought to reflect the intent to mechanically open an artery [redacted].

Because of the difference in frequency of PTCA, and because of the importance of timing with respect to the significance of PCI events, CBER performed time-to-event analyses for the combined PCI variable "stent *or* PTCA" (Figure 11, solid line), and for PTCA alone (Figure 11, broken line).

There was no difference between treatment groups with respect to time-to-event for the "stent or PTCA" variable (log-rank p = 0.53). For PTCA alone, however, there was a significant difference in time-to-event (log-rank P = 0.004), with early separation of the curves (Day 0 through Day 2), and maintenance or slight increase of the separation over time. Thus, whereas PTCA was reported with increased frequency in the TNK group, with a significant difference in time-to-event, the variable reflecting the *intent to revascularize*, "stent or PTCA," had a similar frequency and time course in the TNK and t-PA groups. A plausible (but purely speculative) explanation for the increased frequency of PTCA in the TNK group is that thrombolysis tended to be more complete in this group, decreasing the complexity of the arteriographic appearance of the lesions, such that they were deemed to be more favorable for PTCA alone. This hypothesis can not be tested. Overall, the time-to-event analysis for the combined PCI variable "stent or PTCA" provides reassurance that TNK use was not associated with a greater need for bail-out PCI or subsequent PCI.

Figure 11: Time to PCI Event – Kaplan Meier Curves



Secondary Efficacy Endpoints

Death or Non-Fatal Stroke

Net clinical benefit was defined as death or non-fatal stroke at 30 days. For this endpoint, the sponsor reported an ITT population identical to the ITT population for the primary efficacy endpoint. Thus, there were no subjects with missing stroke status, beyond the 6 subjects with missing 30-day vital status. There were 1198 events observed in the ITT population: 601 and 597 in the TNK group and t-PA groups, respectively.

Reviewer's Comment(s): Upon inspection of the SAS transport file, CBER found 30-day stroke status recorded for all subjects, with none listed as missing. It is of some concern that a negative stroke status is inappropriately reported for the 6 subjects in whom 30-day vital status is missing. This suggests that stroke rates may have been underestimated in both treatment groups.

Using the non-parametric covariate-adjusted method, the relative risk of 30-day mortality or non-fatal stroke for TNK versus accelerated t-PA is 1.017. The upper limit of the one-sided 95% CI (or 2-sided 90% CI) is 1.110, which is within the CBER-proposed upper boundary of 1.14. The unadjusted relative risk was 1.010, with the one-sided upper 95% CI of 1.107 – also within the acceptable limit. Results of the exploratory logistic regression analysis were similar.

Table 42: Death or Non-Fatal Stroke - Relative Risk (Intent to Treat Population)

	TNK			t-PA			RR	90% CI
	n	events	%	n	events	%		
non-parametric adjusted rate								
	8458	601	7.13%	8485	597	7.01%	1.017	(0.931, 1.110)
unadjusted rate								
	8458	601	7.11%	8485	597	7.04%	1.010	(0.921, 1.107)

Exploratory Analysis on the Secondary Endpoint – Underreporting of Strokes

As noted above, the reporting of a non-missing stroke status in 6 subjects who were lost to follow-up raises the concern that some fraction of non-fatal strokes were not appropriately documented and/or transferred to the datasets, such that event rate(s) may be underestimated for one or both groups.

CBER performed a "worst-case" analysis based on the assumption that strokes in the TNK-group were underreported, whereas all strokes in the t-PA group were reported.

For the TNK group, there were 601 events reported: 80 non-fatal strokes and 521 deaths. Assuming that *all* strokes in the t-PA group were reported, CBER calculated that the upper limit of the one-sided 95% CI for relative risk would remain below 1.14 despite the inclusion of as many as 18 additional (hypothetical) non-fatal strokes in the TNK group (i.e., 1 to 18 additional events for a total of 602-619 events). Because an imbalance of this degree in the reporting of non-fatal strokes is unlikely, it is very likely that criteria for the combined death or non-fatal stroke endpoint would have been met, even if non-fatal strokes had been substantially underreported.

Subgroup Analyses

Age, Gender and Race

The 30-day composite of death or non-fatal stroke was similar between the two treatment groups across age, gender and CBER-generated age-gender subgroups (Table 43), as well as for the high-risk subgroups (age > 75 and female gender). With respect to race, although relative risk was essentially unity for Caucasians, t-PA tended to be associated with a better outcome for subjects of African descent (relative risk = 2.95, 95% CI 0.82 – 10.61). Concern regarding the disparity in event rates is tempered by the limited numbers of subjects involved, and also by the fact that the disparity in event rates was due, not to an inordinately high event rate in the TNK group (it was 7.76%, consistent with the TNK group as a whole), but to an unusually low event rate in the t-PA group (2.63%).

The 30-day composite of death or non-fatal stroke tended to decrease with increasing weight, without important disparities between treatment groups.
Reviewer's Comment(s): Because of the interaction between weight and gender, CBER calculated BMI by tertiles and analyzed BMI tertile by gender. For both males and females, death or non-fatal stroke was lowest in the central BMI tertile. No important differences in net clinical benefit between the TNK and t-PA groups were identified in these subgroups.

Table 43: Thirty-Day Composite of Death or Non-Fatal Stroke: Age, Gender, Race, Weight and BMI Subgroups

	TNK			t-PA			RR	95% CI
	n	events	%	n	events	%		
Overall	8458	601	7.11%	8485	597	7.04%	1.010	(0.905, 1.126)
Age (years)								
age <=75	7408	404	5.45%	7410	382	5.16%	1.058	(0.923, 1.212)
age >75	1047	196	18.72%	1070	215	20.09%	0.932	(0.783, 1.109)
*** Age (years)**								
< 53	2282	51	2.23%	2251	45	2.00%	1.118	(0.752, 1.662)
53 - 61	1962	73	3.72%	2025	73	3.60%	1.032	(0.751, 1.419)
62 - 70	2173	158	7.27%	2091	142	6.79%	1.071	(0.86, 1.332)
> 70	2041	319	15.63%	2118	337	15.91%	0.982	(0.854, 1.131)
Gender								
male	6518	378	5.80%	6505	355	5.46%	1.063	(0.923, 1.223)
female	1940	223	11.49%	1980	242	12.22%	0.940	(0.793, 1.116)
*** Gender-Age**								
male; age <= 75	5945	277	4.66%	5915	257	4.34%	1.072	(0.908, 1.266)
male; age > 75	571	100	17.51%	585	98	16.75%	1.045	(0.811, 1.347)
female; age <= 75	1463	127	8.68%	1495	125	8.36%	1.038	(0.82, 1.315)
female; age > 75	476	96	20.17%	485	117	24.12%	0.836	(0.659, 1.061)
Race								
Caucasian	7636	539	7.06%	7637	542	7.10%	0.995	(0.887, 1.116)
African descent	116	9	7.76%	114	3	2.63%	2.948	(0.819, 10.613)
Asian	76	8	10.53%	86	9	10.47%	1.006	(0.409, 2.476)
other	415	37	8.92%	421	28	6.65%	1.341	(0.836, 2.149)
Weight								
< 67	1556	182	11.70%	1575	172	10.92%	1.071	(0.88, 1.303)
>= 67	6874	412	5.99%	6879	420	6.11%	0.982	(0.861, 1.12)
Weight								
< 60	629	86	13.67%	653	83	12.71%	1.076	(0.812, 1.425)
60 - 69	1500	137	9.13%	1479	147	9.94%	0.919	(0.736, 1.147)
70 - 79	2427	161	6.63%	2437	150	6.16%	1.078	(0.869, 1.337)
80 - 89	2061	114	5.53%	2026	127	6.27%	0.882	(0.69, 1.128)
>= 90	1813	96	5.30%	1859	85	4.57%	1.158	(0.871, 1.539)
*** Body Mass Index (BMI) tertile**								
thin	2546	189	7.42%	2540	178	7.01%	1.059	(0.87, 1.29)
medium	2543	145	5.70%	2559	154	6.02%	0.947	(0.76, 1.181)
heavy	2570	129	5.02%	2586	143	5.53%	0.908	(0.72, 1.144)
*** Gender and BMI**								
male - thin	1995	120	6.02%	1939	103	5.31%	1.132	(0.877, 1.462)
male - medium	1959	95	4.85%	1989	103	5.18%	0.936	(0.714, 1.229)
male - heavy	2187	129	5.90%	2251	149	6.62%	0.891	(0.709, 1.12)
female - thin	**551**	**69**	12.52%	**601**	**75**	12.48%	1.003	(0.739, 1.362)
female - medium	584	50	8.56%	570	51	8.95%	0.957	(0.659, 1.389)
female - heavy	**594**	**52**	8.75%	**572**	**55**	9.62%	0.910	(0.634, 1.307)

*** CBER Analyses**

Heart Rate, Blood Pressure, MI Location, Killip Class and Time to Treatment

Tachycardia, hypotension, anterior MI location, advanced Killip class and delayed time to treatment are associated with worse outcome in AMI, and such associations are apparent this study. As observed with respect to mortality, the composite of death or non-fatal stroke is similar between TNK and t-PA groups across these subgroups, with time to treatment the apparent exception. Again, imbalances between the groups with respect to age and baseline blood pressure may partially explain the disparity in death or non-fatal stroke.

Table 44: Thirty-Day Composite of Death or Non-Fatal Stroke – HR, BP, MI Location, Killip Class, Time to Treatment

	TNK			t-PA			RR	95% CI
	n	events	%	n	events	%		
Heart rate (bpm)								
<60	1454	86	5.91%	1456	67	4.60%	1.285	(0.942, 1.754)
60 - 70	1971	104	5.28%	1911	82	4.29%	1.230	(0.927, 1.631)
70 - 80	1900	104	5.47%	1942	134	6.90%	0.793	(0.619, 1.017)
80 - 90	1531	120	7.84%	1500	108	7.20%	1.089	(0.848, 1.398)
90 - 100	815	67	8.22%	837	82	9.80%	0.839	(0.617, 1.142)
> 100	774	118	15.25%	820	120	14.63%	1.042	(0.824, 1.317)
*** Systolic blood pressure (mmHg)**								
< 90	192	58	30.21%	179	50	27.93%	1.081	(0.786, 1.487)
90 - 99	270	33	12.22%	290	42	14.48%	0.844	(0.552, 1.291)
100 - 139	4374	294	6.72%	4321	285	6.60%	1.019	(0.871, 1.193)
140 - 174	3361	196	5.83%	3390	195	5.75%	1.014	(0.836, 1.229)
> 174	251	17	6.77%	293	21	7.17%	0.945	(0.51, 1.751)
MI location								
anterior	3332	303	9.09%	3408	304	8.92%	1.019	(0.876, 1.187)
other	5112	297	5.81%	5059	293	5.79%	1.003	(0.858, 1.173)
Killip Class								
I	7425	422	5.68%	7462	421	5.64%	1.007	(0.884, 1.149)
II	887	129	14.54%	874	122	13.96%	1.042	(0.828, 1.31)
III	93	27	29.03%	98	28	28.57%	1.016	(0.65, 1.587)
IV	35	18	51.43%	36	23	63.89%	0.805	(0.537, 1.207)
Time to treatment (hours)								
< 2	2519	146	5.80%	2563	138	5.38%	1.076	(0.859, 1.35)
> 2 - 4	4036	300	7.43%	3902	254	6.51%	1.142	(0.972, 1.342)
> 4	1832	143	7.81%	1970	200	10.15%	0.769	(0.626, 0.944)

*** CBER Analyses**

Coexistent Diseases; Smoking

Diabetes and prior MI are associated with a poor prognosis in AMI. Death or non-fatal stroke for subgroups with a history of hypertension, diabetes, previous MI, previous CABG and current smoking are shown in Table 45. For subjects with prior CABG, TNK treatment was associated with worse outcome; however, these subjects represented such a small fraction of the overall study population (3.9%) that the meaning is of this observation is subject to question.

Table 45: Thirty-Day Composite of Death or Non-Fatal Stroke – Coexistent Diseases, Current Smoking

	TNK			t-PA			RR	95% CI
	n	events	%	n	events	%		
Hypertension								
No	5263	309	5.87%	5199	310	5.96%	0.985	(0.845, 1.147)
Yes	3187	290	9.10%	3267	283	8.66%	1.050	(0.898, 1.228)
Diabetes								
No	7066	464	6.57%	7140	464	6.50%	1.010	(0.892, 1.144)
Yes	1383	135	9.76%	1329	130	9.78%	0.998	(0.794, 1.254)
Previous MI								
No	7116	454	6.38%	7098	463	6.52%	0.978	(0.863, 1.109)
Yes	1335	146	10.94%	1367	130	9.51%	1.150	(0.919, 1.439)
Previous CABG								
No	8123	564	6.94%	8136	564	6.93%	1.002	(0.895, 1.121)
Yes	327	36	11.01%	327	28	8.56%	1.286	(0.804, 2.056)
*** Smoker (current)**								
No	4622	408	8.83%	4686	410	8.75%	1.009	(0.885, 1.15)
Yes	3744	172	4.59%	3707	158	4.26%	1.078	(0.873, 1.331)

*** CBER Analyses**

Geographic Location; Site Size

Results were consistent across site size tertiles, and generally consistent in the U.S. and Europe. TNK was associated with a trend towards decreased rate of death or non-fatal stroke in Europe, whereas the opposite was true in the U.S. These differences were minor and not of statistical significance.

CBER Exploratory Analyses on Death or Non-Fatal Stroke

Agents with the Potential to Exacerbate Bleeding; TNK/t-PA Excess; Percutaneous Coronary Procedures

Table 46 summarizes relative risk for death or non-fatal stroke for subgroups with use of GPIIb/IIIa agents, LMWH and ticlopidine/clopidogrel, as well as subjects who received > 105% of their "ideal" TNK dose and subjects who underwent PCI. The results parallel those of mortality (Table 40, Table 41). Again, there was a trend towards increased death or non-fatal stroke for subjects in the TNK group who underwent PTCA; however, this appeared to be due, not to an inordinately high event rate in the TNK group, but to an unusually low event rate in the t-PA group; moreover, the numbers of subjects are limited.

Subjects at Low Risk of Cardiac Death

CBER assessed death or non-fatal stroke for subjects at low risk of death from cardiac causes (i.e., males, Killip class I at presentation, no previous history of MI), but at increased risk of stroke (high blood pressure at presentation). For subjects with systolic BP > 139 mmHg, outcomes tended to be worse for the TNK group (Table 47); however, for subjects presenting with more severe hypertension (systolic BP > 175 mmHg), this trend was reversed. Though no definitive conclusions can be drawn from these analyses, the outcomes in the TNK group do not appear to be worse that those in the t-PA group.

Table 46: 30-Day Death or Non-Fatal Stroke - Agents with the Potential to Exacerbate Bleeding; TNK /t-PA Excess; Percutaneous Coronary Interventions

	TNK			t-PA			RR	95% CI
	n	events	%	n	events	%		
*** IIb/IIIa Antagonists**								
No	7801	568	7.28%	7832	566	7.23%	1.008	(0.901, 1.127)
Yes	639	29	4.54%	640	31	4.84%	0.937	(0.572, 1.536)
*** IIb/IIIa agent on Day 0**								
No	8305	591	7.12%	8343	589	7.06%	1.008	(0.903, 1.125)
Yes	153	10	6.54%	142	8	5.63%	1.160	(0.471, 2.857)
*** LMWH use**								
No	6659	541	8.12%	6632	520	7.84%	1.036	(0.923, 1.163)
Yes	1783	55	3.08%	1841	77	4.18%	0.738	(0.525, 1.036)
*** Ticlopidine/Clopidogrel**								
No	6514	563	8.64%	6552	560	8.55%	1.011	(0.904, 1.131)
Yes	1897	33	1.74%	1891	37	1.96%	0.889	(0.558, 1.415)
*** TNK >105% of planned dose (confirmed weights)**								
No	3860	185	4.79%	3894	164	4.21%	1.138	(0.927, 1.398)
Yes	519	14	2.70%	535	12	2.24%	1.203	(0.562, 2.576)
*** weight not confirmed**	4079	402	9.86%	4056	421	10.38%	0.949	(0.834, 1.081)
*** Stent**								
No	6831	560	8.20%	6803	556	8.17%	1.003	(0.896, 1.122)
Yes	1608	38	2.36%	1668	40	2.40%	0.985	(0.636, 1.528)
*** PTCA**								
No	7957	574	7.21%	8065	581	7.20%	1.001	(0.896, 1.119)
Yes	501	27	5.39%	420	16	3.81%	1.415	(0.773, 2.59)
*** Stent or PTCA**								
No	6347	536	8.44%	6396	541	8.46%	0.998	(0.891, 1.119)
Yes	2111	65	3.08%	2089	56	2.68%	1.149	(0.808, 1.634)

*** CBER Analyses**

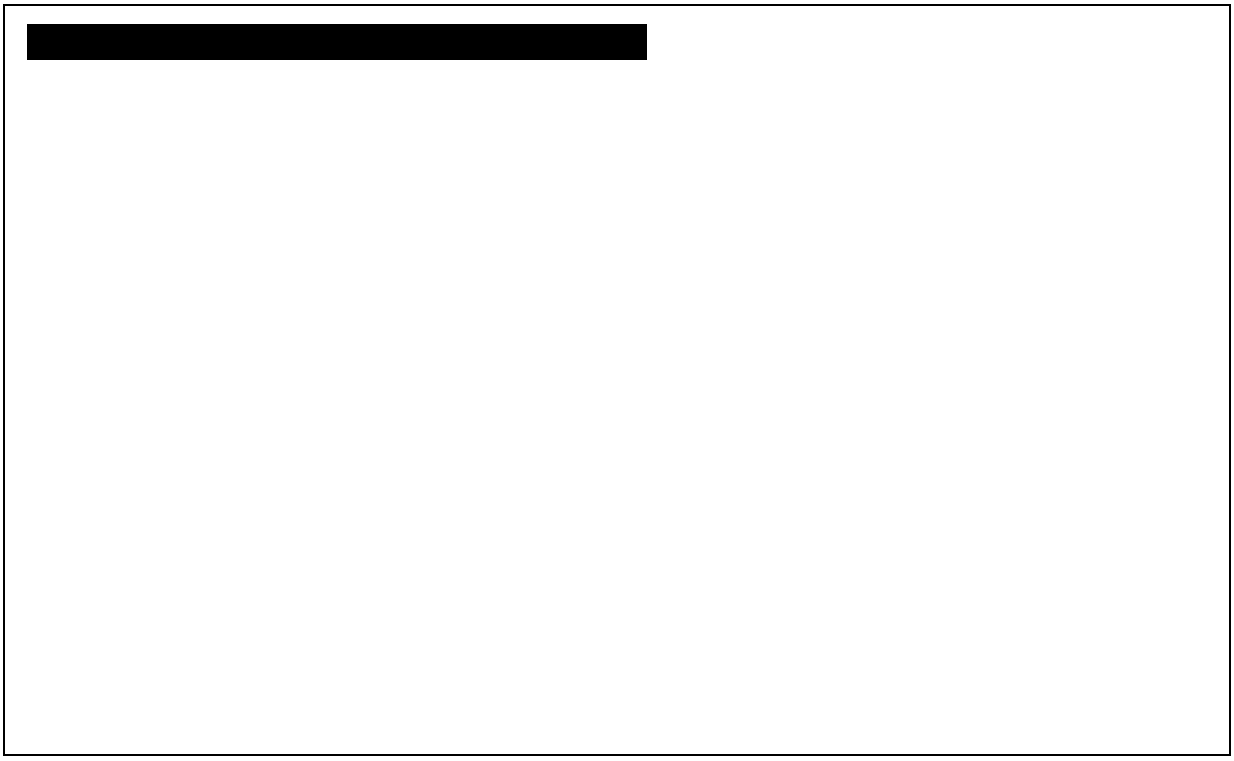
Table 47: Death or Non-Fatal Stroke and Stroke in Subjects with Low Risk of Cardiac Death and High Risk of Stroke (Killip Class I Males, No Prior Hx of MI)

	TNK			t-PA			RR	95% CI
	n	events	%	n	events	%		
BP > 139								
30-day death or non-fatal stroke	2180	85	3.90%	2195	73	3.33%	1.172	(0.862, 1.594)
30-day stroke	2180	38	1.74%	2195	30	1.37%	1.275	(0.793, 2.051)
BP > 175								
30-day death or non-fatal stroke	136	8	5.88%	172	15	8.72%	0.675	(0.295, 1.544)
30-day stroke	136	4	2.94%	172	8	4.65%	0.632	(0.195, 2.056)

Safety Results

In-Hospital Events

The incidences of myocardial re-infarction, sustained hypotension, pulmonary edema, cardiogenic shock, major arrhythmias, pericarditis, acute mitral valve regurgitation, and tamponade were similar in the two treatment groups ([redacted]). The need for invasive procedures (PTCA, stent placement and IABP) was similar between treatment groups. Pulmonary embolism tended to occur more commonly in the TNK group. Surgical revascularization (CABG) was more frequent in the t-PA group; however, considering the multiplicity of comparisons, there is no basis to conclude that this difference was statistically



meaningful.

The sponsor performed exploratory analyses on in-hospital death or non-fatal ICH, death or non-disabling stroke, recurrent angina and acute ventricular septal defect (VSD), and there were no apparent differences between treatment groups.

Killip class was assessed at discharge in the two groups using a Fisher's Exact Test for each Killip category. TNK use was associated with a higher likelihood of Killip I Class status at discharge, and a lower likelihood of Killip Class IV status at discharge. For both groups, these differences were statistically significant. There tended to be fewer TNK-treated subjects in Killip Classes II and III at discharge, though these differences were not statistically significant.

Reviewer's Comment: These were exploratory variables, and the sponsor has appropriately omitted the results of these analyses from the proposed package insert. Nevertheless, the data do suggest improved Killip Class at discharge for the TNK group.

Transient ischemic attacks (TIA), though not defined as a major cardiac event, tended to occur more frequently in the TNK group. There were 25 reported TIA events reported in the TNK group (0.30%) versus 19 (0.22%) reported in the t-PA group (RR = 1.32; 95% CI = 0.73 to 2.40; *p* = 0.37).

The median duration of hospitalization was 8.0 days for each group (Q25–Q75, 5.0–11.0 days

Anaphylaxis

Anaphylaxis tended to occur less commonly with TNK: there were 6 anaphylactic events recorded in the TNK group and 16 in the t-PA group (RR = 0.38; 95% CI = 0.15 to 0.96; *p* = 0.052 - Fisher's Exact Test).

Reviewer's Comment: The proposed label states that "Allergic-type reactions…have rarely been reported in patients treated with TNKase….there are "similar rates of allergic-type reactions with TNKase and Activase." These statements are appropriately conservative.

Strokes

An independent, blinded Stroke Review Panel (SRP) received clinical data and imaging studies and classified strokes as primary hemorrhagic, ischemic, ischemic with hemorrhagic conversion, non-hemorrhagic, unclassifiable or not consistent with stroke. Generally, there was good agreement between investigators' assessments and the SRP (Table 49). Eighteen subjects (11 in the TNK group and 7 in the t-PA group) were placed in the non-classifiable category by the SRP because of insufficient documentation (brain imaging studies and/or autopsy reports). Five subjects had two strokes, and were assigned only to their "worst case" category (i.e., subjects with both primary ICH and non-hemorrhagic stroke were assigned to the ICH category). The reclassification of strokes by the SRP had minimal effect on relative risk for any stroke category, as shown (Table 49).

Table 49: Comparison of Stroke Interpretation by Stroke Review Panel and Investigators

	Stroke Review Panel				Investigators			
	TNK n=8461 events	t-PA n=8488 events	RR	95% CI	TNK n=8461 events	t-PA n=8488 events	RR	95% CI
Total Strokes	**151**	**141**	1.074	(0.86, 1.35)	**153**	**145**	1.074	(0.86, 1.35)
Primary ICH	**79**	**80**	0.991	(0.73, 1.35)	**81**	**90**	0.903	(0.67, 1.22)
Ischemic Stroke	**61**	**54**	1.133	(0.79, 1.63)	**69**	**54**	1.282	(0.90, 1.83)
Unclassified	**11**	**7**	1.576	(0.61, 4.07)	**3**	**1**	3.010	(0.31, 28.93)
No stroke; not consistent with stroke	**5**	**6**			**3**	**2**		

Overall, 292 subjects experienced a stroke: 151 (1.78%) in the TNK group and 141 (1.66%) in the t-PA group (Table 50). Of these subjects, 274 subjects (140 in the TNK group; 134 in the t-PA group) experienced strokes that were classified as primary ICH or ischemic stroke.

Table 50: Total Strokes and Strokes by Stroke Classification

	TNK (n=8461)		t-PA (n=8488)		RR	95% CI
	events	%	events	%		
Total Strokes	151	1.78%	141	1.66%	1.074	(0.856, 1.349)
Primary ICH	79	0.93%	80	0.94%	0.991	(0.727, 1.35)
Ischemic Stroke	61	0.72%	54	0.64%	1.133	(0.787, 1.632)
Hemorrhagic Conversion	6	0.07%	8	0.09%	0.752	(0.261, 2.168)
Unclassified	11	0.13%	7	0.08%	1.576	(0.611, 4.065)

One subject in the TNK group and 4 subjects in the t-PA group had 2 strokes. All five of these subjects had one primary ICH event and one ischemic stroke. (As noted above, all were classified as ICH, and counted only one time for the purpose of these analyses.) Event rates for primary ICH were virtually the same in both groups: 0.93% and 0.94% for the TNK and t-PA groups, respectively. Hemorrhagic conversion of ischemic strokes was reported in 6 subjects (0.07%) in the TNK group and 8 subjects (0.09%) in the t-PA group.

Table 51 and Table 52 present stroke and ICH subgroup results for the ITT population; CBER exploratory analyses are denoted with asterisks.

For both the TNK and t-PA treatment groups, characteristics associated with an increased risk of stroke and primary ICH include age > 75, female gender, weight < 67 kg, female and "thin" BMI tertile, African race and systolic BP > 174 mmHg. Importantly, administration of TNK at > 105% of the ideal dose did not appear to increase the rate of stroke or ICH. Similarly, administration of GPIIb/IIIa agents and/or LMWH did not appear to increase the risk of stroke or ICH.

Event rates for TNK and t-PA were generally similar across all subgroups and for the study as a whole, with respect to both total stroke and primary ICH. With respect to age, there tended to be higher relative rates of stroke (total stroke and ICH) for TNK subjects aged ≤ 75, whereas these trends were reversed for subjects > 75.

In exploratory analyses, CBER assessed rates of stroke and ICH by age in 5 percentile subgroups (Figure 12). Each age group "bin" includes roughly 400 subjects from each treatment group. Rates of stroke appear to increase linearly after the age of 50, with no apparent disparities between treatment groups (Figure 12, left). A similar trend is apparent for ICH (Figure 12, right), although there is greater variability in event rates (the total number of ICH events is only slightly more than half of the stroke event rate,.) Though there is an apparent steep increment in the incidences of ICH events at age ≥ 66 for TNK and age ≥ 72 for t-PA (i.e., a difference of 6 years), the relative infrequency of these events limits the utility of these generalizations.

The incidence of non-hemorrhagic stroke was strikingly high in the African race subgroup of the TNK treatment group (5 events per 116 subjects, 4.3%), compared to no events in the corresponding subgroup of the t-PA treatment arm (0 per 114, 0%). However, given the multiplicity of subgroup analyses, the lack of randomization on this variable, and particularly the limited numbers of subjects in this subgroup, this finding should be interpreted cautiously. Of note, in GUSTO I, the stroke rate for Black subjects was 9/281 = 3.2%.

Table 51: Total Stroke by Major Subgroups – ITT Population

	TNK			t-PA			RR	95% CI
	n	stroke	%	n	stroke	%		
Overall	8461	151	1.78%	8488	141	1.66%	1.074	(0.856, 1.349)
Age (years)								
≤ 75	7408	118	1.59%	7410	94	1.27%	1.256	(0.959, 1.643)
> 75	1047	33	3.15%	1070	47	4.39%	0.718	(0.464, 1.111)
Gender								
male	6518	94	1.44%	6505	78	1.20%	1.203	(0.893, 1.621)
female	1940	57	2.94%	1980	63	3.18%	0.923	(0.649, 1.314)
*** Gender - Age**								
male; age ≤ 75	5945	75	1.26%	5915	52	0.88%	1.435	(1.009, 2.04)
male; age > 75	571	19	3.33%	585	26	4.44%	0.749	(0.419, 1.337)
female; age ≤ 75	1463	43	2.94%	1495	42	2.81%	1.046	(0.688, 1.591)
female; age > 75	476	14	2.94%	485	21	4.33%	0.679	(0.35, 1.32)
Weight								
< 67	1556	45	2.89%	1575	48	3.05%	0.949	(0.636, 1.417)
≥ 67	6874	106	1.54%	6879	93	1.35%	1.141	(0.865, 1.504)
*** BMI and Gender**								
thin - male	1995	39	1.95%	1939	24	1.24%	1.579	(0.954, 2.616)
medium - male	1959	24	1.23%	1989	26	1.31%	0.937	(0.54, 1.627)
heavy - male	2187	26	1.19%	2251	30	1.33%	0.892	(0.529, 1.503)
thin - female	551	18	3.27%	601	26	4.33%	0.755	(0.419, 1.362)
medium - female	584	12	2.05%	570	13	2.28%	0.901	(0.415, 1.958)
heavy - female	594	19	3.20%	572	12	2.10%	1.525	(0.747, 3.112)
Race								
Caucasian	7636	136	1.78%	7637	134	1.75%	1.015	(0.801, 1.286)
African descent	116	7	6.03%	114	1	0.88%	6.879	(0.86, 55.028)
Asian	76	3	3.95%	86	0	0.00%		
other	415	4	0.96%	421	3	0.71%	1.353	(0.305, 6.007)
*** Systolic blood pressure (mmHg)**								
< 90	192	4	2.08%	179	4	2.23%	0.932	(0.237, 3.672)
90 - 99	270	6	2.22%	290	8	2.76%	0.806	(0.283, 2.292)
100 - 139	4374	65	1.49%	4321	55	1.27%	1.167	(0.817, 1.668)
140 - 174	3361	67	1.99%	3390	63	1.86%	1.073	(0.763, 1.508)
> 174	251	9	3.59%	293	10	3.41%	1.051	(0.434, 2.545)
Time to treatment (hours)								
< 2	2519	35	1.39%	2563	27	1.05%	1.319	(0.801, 2.172)
> 2 - 4	4036	81	2.01%	3902	70	1.79%	1.119	(0.815, 1.536)
> 4	1832	33	1.80%	1970	43	2.18%	0.825	(0.527, 1.293)
*** IIb/IIIa Antagonists**								
No	7801	144	1.85%	7832	136	1.74%	1.063	(0.843, 1.341)
Yes	639	7	1.10%	640	5	0.78%	1.402	(0.447, 4.395)
*** LMWH use**								
No	6659	134	2.01%	6632	123	1.85%	1.085	(0.851, 1.383)
Yes	1783	17	0.95%	1841	18	0.98%	0.975	(0.504, 1.886)
*** TNK >105% of planned dose**								
No	3860	63	1.63%	3894	51	1.31%	1.246	(0.864, 1.798)
Yes	519	8	1.54%	535	4	0.75%	2.062	(0.625, 6.805)
weight unconfirmed	4079	80	1.96%	4056	86	2.12%	0.925	(0.684, 1.25)

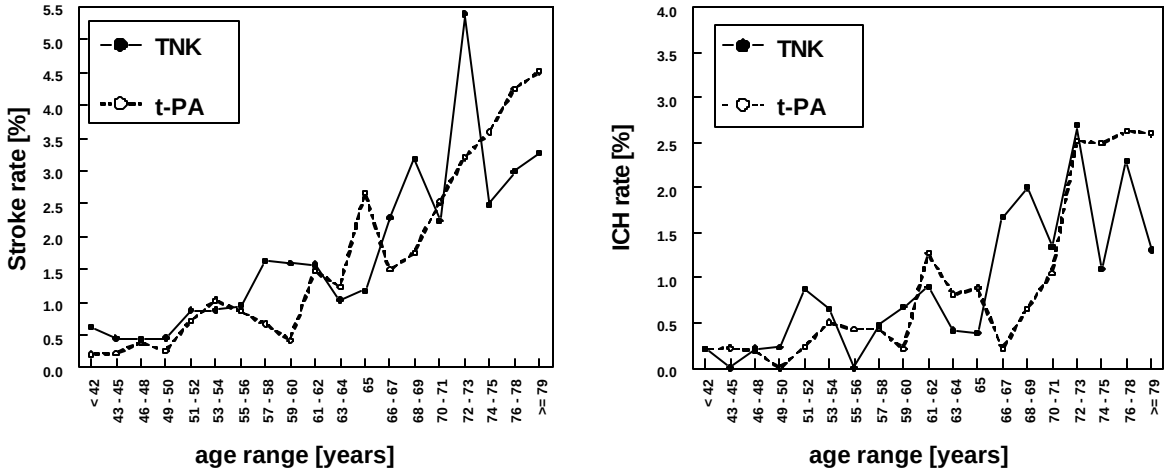
*** CBER Analyses**

Table 52: Primary ICH by Major Subgroups – ITT Population

	TNK			t-PA			RR	95% CI
	n	ICH	%	n	ICH	%		
Overall	8461	79	0.93%	8488	80	0.94%	0.991	(0.727, 1.35)
Age (years)								
≤ 75	7408	61	0.82%	7410	52	0.70%	1.173	(0.812, 1.696)
> 75	1047	18	1.72%	1070	28	2.62%	0.657	(0.366, 1.18)
Gender								
male	6518	51	0.78%	6505	45	0.69%	1.131	(0.759, 1.686)
female	1940	28	1.44%	1980	35	1.77%	0.816	(0.499, 1.337)
*** Gender - Age**								
male; age ≤ 75	5945	39	0.66%	5915	29	0.49%	1.338	(0.829, 2.161)
male; age > 75	571	12	2.10%	585	16	2.74%	0.768	(0.367, 1.61)
female; age ≤ 75	1463	22	1.50%	1495	23	1.54%	0.977	(0.547, 1.746)
female; age > 75	476	6	1.26%	485	12	2.47%	0.509	(0.193, 1.346)
Weight								
< 67	1556	22	1.41%	1575	28	1.78%	0.795	(0.457, 1.384)
≥ 67	6874	57	0.83%	6879	52	0.76%	1.097	(0.754, 1.595)
*** BMI and Gender**								
thin - male	1995	16	0.80%	1939	17	0.88%	0.915	(0.464, 1.805)
medium - male	1959	15	0.77%	1989	11	0.55%	1.385	(0.638, 3.007)
heavy - male	2187	18	0.82%	2251	14	0.62%	1.323	(0.66, 2.654)
thin - female	551	10	1.81%	601	15	2.50%	0.727	(0.329, 1.605)
medium - female	584	4	0.68%	570	8	1.40%	0.488	(0.148, 1.612)
heavy - female	594	9	1.52%	572	7	1.22%	1.238	(0.464, 3.302)
Race								
Caucasian	7636	72	0.94%	7637	75	0.98%	0.960	(0.696, 1.325)
African descent	116	2	1.72%	114	1	0.88%	1.966	(0.181, 21.377)
Asian	76	1	1.32%	86	0	0.00%		
other	415	3	0.72%	421	1	0.24%	3.043	(0.318, 29.14)
*** Systolic blood pressure (mmHg)**								
< 90	192	2	1.04%	179	1	0.56%	1.865	(0.171, 20.387)
90 - 99	270	3	1.11%	290	2	0.69%	1.611	(0.271, 9.568)
100 - 139	4374	26	0.59%	4321	33	0.76%	0.778	(0.466, 1.299)
140 - 174	3361	42	1.25%	3390	36	1.06%	1.177	(0.756, 1.832)
> 174	251	6	2.39%	293	8	2.73%	0.875	(0.308, 2.489)
Time to treatment (hours)								
< 2	2519	24	0.95%	2563	20	0.78%	1.221	(0.676, 2.205)
> 2 - 4	4036	34	0.84%	3902	40	1.03%	0.822	(0.521, 1.295)
> 4	1832	20	1.09%	1970	20	1.02%	1.075	(0.58, 1.992)
*** IIb/IIIa Antagonists**								
No	7801	75	0.96%	7832	79	1.01%	0.953	(0.696, 1.305)
Yes	639	4	0.63%	640	1	0.16%	4.006	(0.449, 35.747)
*** LMWH use**								
No	6659	76	1.14%	6632	75	1.13%	1.009	(0.735, 1.386)
Yes	1783	3	0.17%	1841	5	0.27%	0.620	(0.148, 2.589)
*** TNK >105% of planned dose**								
No	3860	27	0.70%	3894	26	0.67%	1.048	(0.613, 1.792)
Yes	519	4	0.77%	535	3	0.56%	1.374	(0.309, 6.111)
weight unconfirmed	4079	48	1.18%	4056	51	1.26%	0.936	(0.633, 1.385)

*** CBER Analyses**

Figure 12: Rates of Stroke and ICH by Age, TNK and t-PA



Event rates were similar between the two treatment groups with respect to in-hospital, non-fatal, disabling strokes (Grade 2-5 on the Modified Rankin Scale), both for primary ICH and for non-ICH strokes (data not shown).

Non-ICH Bleeding Events

The most common complication encountered during thrombolytic therapy is bleeding. For subjects with > 1 bleed during the in-hospital period, the subject was counted once according to the worst event recorded. Bleeding events reported in this section do not include primary ICHs. Bleeding was recorded only during the in-hospitalization phase of the trial because it was assumed that the bleeding risk due to the thrombolytic was reduced to zero at discharge.

Overall, there were fewer bleeding events in the TNK group than the t-PA group. In-hospital bleeding events (all events, uncharacterized) were reported in 26.4% and 28.9% of subjects in the TNK and t-PA groups, respectively (Table 53). There were also significantly fewer major bleeding events in the TNK group: major bleeding was reported in 4.66% and 5.94% of subjects in the TNK and t-PA groups, respectively. These results were consistent across all body weight classes (data not shown). Site-specific bleeding is listed in Table 53 by decreasing frequency of event. With the exception of oropharyngeal bleeds, there tended to be less bleeding associated with TNK treatment than t-PA treatment.

Transfusions

Overall, approximately 5% of subjects required blood transfusions during the in-hospital study period (Table 53). Of the 776 subjects who required transfusions, 460 required 1–2 units, 308 required > 2 units, and the numbers of units was unrecorded for 8 subjects. Blood transfusions were reported less frequently in the TNK group (4.25%) than in the t-PA group (5.49%), with the disparity paralleling the difference observed for major bleeding.

Serious, Unexpected, Study Drug-Related Adverse Events

During the study, 22 serious, unexpected, study drug–related adverse events occurred: 18 in the TNK group and 4 in the t-PA group. Events in the TNK group included: myocardial rupture (5 subjects), ischemic/ embolic stroke (4 subjects), ventricular septal defect (VSD) (3 subjects),

Table 53 : Non-ICH Bleeding Events, ITT Population

	TNK			t-PA		
	n	events	%	n	events	%
Bleeding by Severity						
All Bleeds	8461	2236	26.43%	8488	2457	28.95%
Major Bleeds	8461	394	4.66%	8485	504	5.94%
Serious Bleeds	8458	79	0.93%	8485	99	1.17%
Severe Bleeds	8461	64	0.76%	8486	86	1.01%
Mild or Moderate Bleeds	8460	2171	25.66%	8486	2369	27.92%
Bleeding by Site						
Hematoma	8461	716	8.46%	8488	776	9.14%
Venous Puncture Site Bleed	8461	638	7.54%	8488	730	8.60%
GU Bleed	8461	348	4.11%	8488	422	4.97%
Arterial Puncture Site Bleed	8461	348	4.11%	8488	387	4.56%
GI Bleed	8461	241	2.85%	8488	283	3.33%
Oropharyngeal Bleed	8461	277	3.27%	8488	247	2.91%
Intracranial Bleed (non-ICH)	8461	14	0.17%	8488	23	0.27%
Retroperintoneal Bleed	8461	13	0.15%	8488	11	0.13%
Units Transfused Blood						
none	7881	7546	95.75%	7890	7457	94.51%
1 - 2	7881	204	2.59%	7890	256	3.24%
> 2	7881	131	1.66%	7890	177	2.24%

cardiogenic shock (2 subjects), jaundice (1 subject), infarction of spinal conus medullaris (1 subject), rhabdomyolysis (1 subject) and worsening of peripheral vascular disease (1 subject). Events in the t-PA group included: cerebral hypoperfusion and/or seizures (1 subject), cerebral infarction (1 subject), electromechanical dissociation and cardiogenic shock (1 subject) and rhabdomyolysis (1 subject).

Reviewer's Comment(s): The classification of these events as serious, unexpected and study drug-related is suspect. For example, 4 strokes were categorized as serious, unexpected and drug-related, yet there were roughly 300 strokes in the study overall. There were 3 instances of ventricular septal defect (VSD) reported as serious, unexpected and drug related, yet acute VSD was reported in 22 and 27 subjects in the TNK and t-PA groups, respectively.

These events are known to occur in an AMI patient population, albeit at an infrequent rate. Their frequencies in this study appear to be in line with prior experience, and are generally consistent between treatment groups. Two types of complications merit discussion:

- The more catastrophic cardiac complications of AMI, including myocardial rupture, tamponade, acute mitral regurgitation and the need for intra-aortic balloon pump insertion, occurred with slightly greater frequency in the t-PA group, providing some measure of reassurance that these events are not *more frequent* with TNK.

- AMI patients are known to have a thrombotic diathesis. This was particularly apparent in the years prior to the widespread use of thrombolytics and heparin. Given that bleeding and thrombosis are on the opposite ends of the hemostatic spectrum, and in light of the fact that there appeared to be excess bleeding in the t-PA group, it is interesting that there were trends toward higher incidences of thrombotic events in the TNK group. Specifically, there were 8 reports of pulmonary embolism in the TNK group, as compared to 3 in the t-PA group. There were 25 reports of transient ischemic attack (TIA) in the TNK group, and 19 in the t-PA group. These event rates are small and no definitive conclusions are possible; however, these data, in conjunction with the bleeding data, suggest a decreased state of systemic thrombolysis with TNK relative to t-PA, at the doses used in this study.

Adverse Events

Adverse events were reported in 74.5% and 75.5% of subjects in the TNK and t-PA groups, respectively. Adverse events classified by system organ class included: application-site disorders (1.4% in both groups); cardiovascular disorders (general) (21% in both groups); heart rate and rhythm disorders (42% in both groups); liver and biliary system disorders (TNK 0.3%, t-PA 0.4%); metabolic and nutritional disorders (TNK 3.4%, t-PA 3.9%); platelet, bleeding and clotting disorders (TNK 23.5%, t-PA 25.4%); respiratory symptom disorders (TNK 15.6%, t-PA 16.2%); and urinary system disorders (TNK 7.7%, t-PA 8.8%). The overall safety profile did not differ from other large-scale clinical trials in AMI patients.

Conflicts of Interest

In accordance with 21 CFR Part 54, Financial Disclosure by Clinical Investigators, the sponsor attempted to obtain statements of financial disclosure from investigators in ASSENT II. Of the thousands of investigators involved in ASSENT II, only two disclosed potential conflicts of interest [redacted]. Many investigators required to disclose potential conflicts of interest disclosed no conflicts; however, there was a very substantial number of investigators who did not respond to the sponsor's requests for disclosure.

CBER performed an exploratory analysis based on the "worst-case" assumption - - that investigators who did not respond to requests for disclosure are, in fact, hiding conflicts of interest, and that entire study sites of such investigators exhibited bias in favor of TNK. In this scenario, 46% of subjects were considered to be enrolled at sites with conflicts of interest. For the mortality, ICH and stroke endpoints, the results were consistent between subjects at sites with and without conflicts of interest, and suggest that conflicts of interest, if present, had no material effect on the results of ASSENT II.

ASSENT II Summary

ASSENT II was one of the larger international trials of a thrombolytic agent, an active-control, non-inferiority study comparing the efficacy and safety of bolus TNK to accelerated t-PA in subjects with AMI presenting within 6 hours of symptom onset. A total of 17,005 subjects were enrolled at 1,022 sites in 29 countries in North and South America, Europe, Africa and Australia. Subjects were randomized 1:1 to TNK and t-PA. Thirty-day vital status was obtained for all but 3 subjects in each group. All cause mortality totaled 1045, with 521 and 524 deaths in the TNK and t-PA groups, respectively. The unadjusted mortality rates, rounded to a tenth of a percent, were identical: 6.2% in each group. Unadjusted relative risk was 0.997, with 95% CI 0.887 to 1.122. Thus, irrespective of the test used for non-inferiority, and irrespective of the fate of any subjects lost to follow-up, all cause mortality was essentially identical in the two treatment groups.

There were 159 recorded ICH events: 79 events were reported in the TNK group (TNK rate = 0.93%); 80 events were reported in the t-PA group (t-PA rate = 0.94%, relative risk 0.991, 95% CI 0.73 to 1.35). Total strokes numbered 292: 151 in the TNK group and 141 in the t-PA group. Respective rates were 1.8% and 1.7%, relative risk = 1.074, 95% CI 0.856 to 1.349.

Several interesting trends emerged from the data:

- There was a trend towards a mortality advantage for TNK in subjects treated between 4 to 6 hours after symptom onset.
- There appeared to be less bleeding in the TNK group; conversely, there was a trend towards excess thrombotic events in the TNK group (pulmonary embolism and transient ischemic attack).
- Total strokes numbered 8 in the 230 subjects of African descent; however, 7 of 8 events were reported in the TNK group, suggesting differential effects of TNK and t-PA in this minority population.

The strength and validity of these observations are limited by the post hoc nature and multiplicity of analyses; nevertheless, they are interesting as hypotheses and have significant public health ramifications.

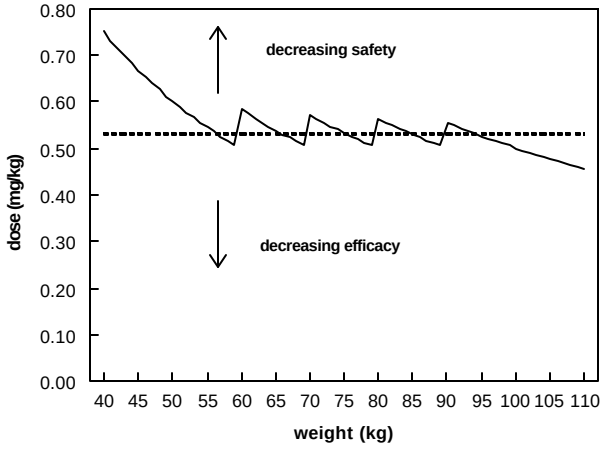
Overall, the study supports the contention that TNK is not inferior to native t-PA in efficacy or safety in the treatment of AMI subjects presenting within 6 hours of symptom onset.

CBER Analyses of Stepped Dosing Paradigm

Though the phase 2 studies suggested that weight-adjusted dosing would provide an optimal risk-benefit relation for TNK, there was no scientific rationale for the stepped dosing paradigm, per se. By design, subjects with weights rounded to the whole number and ending in numerals 4, 5 and 6 receive weight-adjusted doses close to the target of 0.53 mg/kg. Subjects with weights ending in digits 0, 1, 2 and 3 receive higher doses; subjects with weights ending in digits 7, 8 and 9 receive lower doses. Moreover, because the stepped dosing regimen encompasses a finite range, subjects at the lower and upper extremes of weight (< 50 or > 100 kg) receive progressively higher and lower weight-adjusted doses.

The concern resulting from this weight-adjusted dose versus weight function relates to the "therapeutic window" of TNK. Specifically, if small differences in weight-adjusted dose bear importantly on efficacy and/or safety, then the stepped paradigm may lead to an unfavorable risk-benefit relation in certain subgroups, depending on weight. On the other hand, if moderate or large disparities between the actual and optimal weight-adjusted doses have minimal impact on efficacy or safety, then the stepped dosing approach is acceptable with respect to efficacy and safety.

Figure 13: Effect of Weight on Weight-Adjusted TNK Dose



CBER performed two principal analyses to address this issue: 1) an analyses of efficacy and

safety by weight-adjusted dose, and 2) for subjects in whom actual weights were not recorded, an assessment of the effect of estimated weight on TNK efficacy and safety.

Relation Between Weight-Adjusted Dose, Efficacy and Safety

CBER combined TNK subjects from ASSENT I and ASSENT II, including from ASSENT I only subjects who were enrolled subsequent to the protocol changes impacting on heparin and abciximab administration guidelines. For these 11,112 subjects, actual weights were recorded in 7040 (63%). Major clinic events (death, ICH, stroke and serious bleeding events) were tabulated by weight-adjusted dose sextiles, (Table 54), and by arbitrary tiers of weight-adjusted dose (0.05 mg/kg below the 0.53 target; 0.04 mg/kg above the 0.53 target, Table 55). The purpose of the latter tabulation was to critically examine event rates at the high extreme of weight-adjusted dose.

Of note, there were excess deaths and tended to be excess ICH events in subjects in whom weight was not recorded, possibly because of worse clinical status on presentation.

Table 54: ASSENT I and ASSENT II – Rates of Major Clinical Events by Weight-Adjusted Dose Sextile - Subjects Who Received TNK

Weight-adjusted dose stratum (mg/kg)	N	Major Clinical Events (n, %)			
		Death	ICH	Stroke	Serious Bleeding Event
No weight	4072	410 (10.1)	53 (1.3)	86 (2.1)	41 (1.0)
< 0.42	1172	37 (3.2)	3 (0.3)	12 (1.0)	23 (2.0)
0.42 to < 0.50	1126	48 (4.3)	11 (1.0)	16 (1.4)	21 (1.9)
0.50 to < 0.53	1189	42 (3.5)	7 (0.6)	17 (1.4)	15 (1.3)
0.53 to < 0.55	1206	47 (3.9)	8 (0.7)	20 (1.7)	17 (1.4)
0.55 to < 0.57	1151	40 (3.5)	6 (0.5)	16 (1.4)	11 (1.0)
>= 0.57	1196	52 (4.3)	12 (1.0)	24 (2.0)	27 (2.3)

Table 55: ASSENT I and ASSENT II – Rates of Major Clinical Events by Weight-Adjusted Dose - Subjects Who Received TNK (subgroups divided by 0.04 to 0.05 mg/kg brackets)

Weight-adjusted dose stratum (mg/kg)	N	Major Clinical Events (n, %)			
		Death	ICH	Stroke	Serious Bleeding Event
No weight	4072	410 (10.1)	53 (1.3)	86 (2.1)	41 (1.0)
< 0.48	1959	71 (3.6)	10 (0.5)	23 (1.2)	41 (2.1)
0.48 to < 0.53	1883	71 (3.8)	14 (0.7)	28 (1.5)	21 (1.1)
0.53 to < 0.57	2040	72 (3.5)	11 (0.5)	30 (1.5)	25 (1.2)
0.57 to < 0.61	706	29 (4.1)	7 (1.0)	14 (2.0)	15 (2.1)
0.61 to < 0.65	229	11 (4.8)	1 (0.4)	2 (0.9)	8 (3.5)
> 0.65	223	12 (5.4)	4 (1.8)	8 (3.6)	4 (1.8)

In Table 55, event rates appear fairly uniform across all dose/weight sextiles. For the most extreme weight-adjusted dose cohort (Table 55, bottom), there were trends towards excess ICH and strokes, but paradoxically no apparent increase in the rate of serious bleeding events. These findings, though suggestive of decreased safety at the highest weight-adjusted dose, are not conclusive considering the small number of subjects and the low numbers of events in this subgroup.

Finally, CBER performed an exploratory analysis to assess the effect of weight estimation on efficacy and safety. Included in this analysis were subjects in ASSENT II who actually received TNK, who had no recorded actual weight, and whose TNK dose was guided by an estimated weight (n =3861). The incidences of major clinical events are summarized by ending digit of estimated weight (Table 56).

As expected, there is a major peak in subject number for digits 0 and 1, with a secondary peak for digits 4 and 5 (reflecting the tendency to estimate weights to the nearest 10 and 5 kg). As noted above, the overall mortality rate in this group is higher than that for subjects with recorded weights. Nevertheless, there do not appear to be disparities for different "digit" subgroups. Similarly, rates of ICH, stroke and serious bleeding events appear similar. Thus, although the inability to obtain an actual weight appears to be associated with a worse prognosis, the actual numerical weight guessed, a critical factor for selection

Table 56: ASSENT I and ASSENT II – Rates of Major Clinical Events in Subjects Without Recorded Weights: Effect of Last Digit of Estimated Weight

		Major Clinical Events (n, %)			
ending digit of estimated weight	N	Death	ICH	Stroke	Serious Bleeding Event
0 or 1	1130	111 (9.8)	15 (1.3)	23 (2.0)	14 (1.2)
2 or 3	644	52 (8.1)	10 (1.6)	14 (2.2)	6 (0.9)
4 or 5	973	76 (7.8)	7 (0.7)	13 (1.3)	4 (0.4)
6 or 7	530	54 (10.2)	8 (1.5)	14 (2.6)	5 (0.9)
8 or 9	584	51 (8.7)	8 (1.4)	14 (2.4)	4 (0.7)

of the dosing tier, does not appear to importantly affect outcome.

Summary and Recommendations

In summary, these studies adequately support the safety and efficacy of TNK in the treatment of AMI subjects presenting within 6 hours of symptom onset. Using t-PA as a comparator, the phase 2 TIMI 10B study provides clear evidence of the thrombolytic activity of the product at higher doses. By the prospective criteria invoked to establish sufficiency of retained benefit, the pivotal phase 3 study supports the contention that TNK retains sufficient effectiveness and safety relative to native t-PA in the treatment of AMI. Of note, unlike the marketed thrombolytic agents, the effect of the product on left ventricular function was not systematically assessed in the development plan, and is not a labeling claim.

In general, the safety profile of TNK appears similar to that of t-PA. Data from ASSENT II suggest that TNK, at the dose studied, was associated with fewer bleeding events than t-PA, while associated with a greater frequency of thrombotic events. Given that thrombolysis and thrombosis are at opposing extremes of a pathophysiologic continuum, it is possible that the

balance between bleeding and thrombosis might have been shifted (and more closely matched to t-PA) had a different weight-adjusted TNK dose been used in the studies. These differences with respect to bleeding and thrombosis are minor however, and are not of sufficient magnitude to raise concern.

There was concern regarding the weight-adjusted stepped dosing paradigm used in ASSENT II. By design, patients with weights < 50 kg received weight-adjusted TNK doses progressively higher than the target dose of 0.53 mg/kg; conversely, patients with weights > 100 kg received weight-adjusted TNK doses progressively less than 0.53 mg/kg. Also by design, patients whose weights were rounded up to the nearest multiple of 10 kg were shifted to a higher dosing tier. CBER's analyses suggest that efficacy and safety were not compromised by this scheme, although the experience in low-weight subjects was too limited to draw meaningful conclusions. The necessity to estimate a subject's weight in ASSENT II was associated with worse outcome (relative to subjects in whom actual weights were recorded); however, the actual numerical weight guessed, a critical factor for assignment to a dosing tier, did not appear to importantly affect mortality or critical aspects of safety.

Anaphylaxis was uncommon in TNK-treated subjects, and appeared to occur no more commonly than in t-PA-treated subjects. The antibody response to the product was evaluated in TIMI 10A and TIMI 10B, in which a total of 487 subjects were adequately assessed. One subject had a positive titer at 30 days, that was subsequently negative at 90 days.

Key remaining concerns regarding safety of TNKase could be addressed in phase 4 commitments:

1. Subjects of African descent were underrepresented in the studies in proportion to their representation in the U.S. population at large, and there appeared to be a disproportionate rate of strokes in subjects of African descent who received TNK.

2. The safety of TNK is not well characterized in Hispanic subjects. This demographic characteristic was not included in the case report form for ASSENT II.

3. The stepped dosing paradigm leads to weight-adjusted doses that become progressively higher in patients below 50 kg in weight. Data are limited in this subgroup.

4. Anti-TNKase antibody data were available for fewer than 500 subjects in TIMI 10A and TIMI 10B, and collection of additional data may be warranted to substantiate safety.

5. There was a trend towards improved outcome in TNK-treated subjects treated between 4 and 6 hours after symptom onset. This observation does not raise a critical safety or efficacy concern; however, the sponsor should be encouraged to construe this as a generated hypothesis that may merit additional study, because of the ramifications for public health.

With the above provisions, approval is recommended.